Regulation of Dendritic Cell Function by Nutrients

der Fakultät für Biologie
der EBERHARD KARLS UNIVERSITÄT TÜBINGEN
zur Erlangung des Grades eines Doktors
der Naturwissenschaften

von

NguyenThi Xuan
aus Thai Binh, Vietnam

vorgelegte

DISSERTATION

2009

Tag der mündlichen Prüfung: 02.11.2009

Dekan: **Prof. Dr. Hanspeter Mallot**

1. Berichterstatter: **Prof. Dr. Florian Lang**

2. Berichterstatter: **Prof. Dr. Fritz Götz**

ACKNOWLEDGEMENTS

It is with immense pleasure, I am thankful very much to my research guide Prof. Dr. Florian Lang, for his scientific guidance, critical review, support throughout my Ph.D. work at Institut of Physiology, University of Tübingen, Germany.

I am very grateful to Dr. Shumilina Ekaterina for her timely help, support, thought provoking suggestions and stimulating discussions during the progress of the work. I would like to thank Prof. Dr. Fritz Götz for giving me an opportunity to present the dissertation at the Faculty of Biology, Eberhard Karls Universität Tübingen, Germany.

I am very thankful to my friends and my colleagues Ms. Matzner Nicole, Dr. Zemtsova Irina, Mr. Mahmud Hasan, Mr Diwakar Bobbala, Mr Qadri Syed for their help, suggestions, friendship and support. I also thanks to my colleagues Ms. Zhang Ying, Mrs. Efi Faber, Mr. Uwe Schueler, Mr. Rexhepaj Rexhep and Mr. Peter for their support.

This task never completes without the mention of my loving parents, my brother and sister who have blessed me in every aspect of my life.

I dedicate my thesis to my husband Dr. Quach Duc Tin and my daughter Quach Huong Linh for being a constant source of inspiration for my work. I am greatly indebted for his support, constructive criticism and endless love in my life.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii
TABLE OF CONTENTS iv
ABBREVIATIONS viii
ABSTRACT x
ZUSAMMENFASSUNG xi
1. INTRODUCTION 1
1.1. Adaptive immune system and innate immunity 1
1.1.1. Innate immune system 1
1.1.2. Adaptive immune system 2
1.2. Nature of DCs 4
1.3. Interaction of DCs with NK, B and T cells 6
1.4. Tolerance immunity and Th1/Th2 balance drived by DCs 7
1.5. Toll-like receptors (TLRs) 8
1.6. Subsets of conventional mouse DCs 9
1.7. Migration and Phagocytosis 11
1.8. The mucosal immune system 11
1.9. Mucosal dendritic cells 12
1.10. Cell death 13
1.10.1. Intrinsic apoptosis pathway 13
1.10.2. Extrinsic apoptosis pathway 14
1.10.3. Sphingomyelinase pathway 14
1.10.4. Regulation of apoptosis by ceramide 15
1.10.5. The Bcl-2 family: inhibitors and promoters of apoptosis 15
1.10.6. Caspase activity 16
1.11. Nutrients 16
1.11.1. Thymoquinone 17
1.11.2. Gum Arabic 17
1.11.3. Zn^{2+} 18
1.11.4. Xanthohumol 19
1.11.5. Thymol 19
2. AIMS OF THE STUDY 20
3. MATERIAL AND METHODS 20
3.1. Equipment 20
3.2. Mice 21
3.3. Chemicals 21
3.4. DC culturing 22
3.5. Immunostaining and flow cytometry 23
3.6. Cytokine quantification in cell supernatants 23
3.7. DC phagocytosis assay 23
3.8. Immunoblotting 24
3.9. Isolation of T and B lymphocytes from the spleen 24
3.10. Phosphatidyl residue translocation 25
3.11. Ceramide formation 25
3.12. DNA fragmentation 25
3.13. Measurements of mitochodrial membrane potential 26
3.14. Caspase 8 and Caspase 3 activation assay 26
3.15. Immunocytochemistry 26

3.16. Statistics 27
4. RESULTS 27
4.1. Thymoquinone 27
4.1.1. Thymoquinone inhibits maturation of LPS- stimulated DCs. 27
4.1.2. Thymoquinone impairs cytokine secretion by LPS- stimulated DCs 29
4.1.3. Thymoquinone enhances percentage of annexin V-binding DCs. 30
4.2. Gum Arabic 31
4.2.1. GA enhances bone marrow-derived DC differentiation and maturation 31
4.2.2. GA enhances cytokine secretion in DCs 34
4.2.3. GA decreases phagocytic capacity in DCs 35
4.2.4. Stimulation with GA leads to upregulation of extracellular signal-regulated kinase (ERK1 and ERK2) in DCs 36
4.2.5. GA treatment leads to a higher percentage of splenic $CD8^+$ T cells and B cells 38
4.3. Zn^{2+}, xanthohumol and thymol 39
4.3.1. Effect of Zn^{2+}, xanthohumol and thymol on ceramide formation 39
4.3.2. Effect of Zn^{2+}, xanthohumol and thymol on DNA fragmentation 43
4.3.3. Effect of xanthohumol and thymol on caspase 8 activity 45
4.3.4. Effect of xanthohumol and thymol on caspase 3 activity 47
4.3.5. Effect of Zn^{2+}, xanthohumol and thymol on cell membrane scrambling 49
4.3.6. Effect of C2-ceramide on DC apoptosis 51
4.3.7. Effect of Zn^{2+} and thymol on Bcl-2 and Bcl-xL protein abundance 52
4.3.8. Effect of xanthohumol on mitochodrial menbrane potential of DCs 54
4.3.9. Xanthohumol and thymol impaired and Zn^{2+} enhanced cytokine secretion by LPS-stimulated DCs. 56
5. DICUSSION 58
5.1. Thymoquinone 59
5.2. Gum Arabic 59
5.3. Zn^{2+} 60
5.4. Xanthohumol 61
5.5. Thymol 62
6. REFERENCES 64
CURICULUM VITAE 84
PUBLICATIONs 86

LIST OF FIGURES

Introduction

Figure A: Antigen presentation stimulates T cells to become either "cytotoxic" $CD8^+$ cells or "helper" $CD4^+$ cells. 3

Figure B: DC biology: differentiation, migration, antigen presentation, and tolerance. (Harizi and Gualde 2005)..... 19

Figure C: Schematic representation of DC-innate lymphocyte cross-talk in the activation of the immune response (Reschner et al 2008b)..... 6

Figure D: TLR-mediated immune responses (Takeda and Akira 2005b)..... 9

Figure E: Signalling through sphingomyelinase pathway..... 14

Results

Figure 1: Effect of thymoquinone on LPS-induced CD86, MHC class II and CD40 surface expression in mouse DCs 28

Figure 2: Effect of thymoquinone on LPS-induced CD54 surface expression in mouse DCs 29

Figure 3: Effect of thymoquinone on LPS-induced formation of IL-10, IL-12 and TNF-α in mouse DCs 30

Figure 4 31

Figure 5: Effect of LPS and GA treatment on MHC class II, CD86 and CD40 surface expression in DCs..... 32

Figure 6: Effect of LPS and GA treatment on CD54 surface expression in DCs 33

Figure 7: Effect of LPS and GA treatment on the formation of IL-10, IL-12p70, IL-6 and TNF-α 34

Figure 8: LPS and GA decrease phagocytic capacity of DCs..... 35

Figure 9: GA enhances the phosphorylation of ERK1 and ERK2 in DCs..... 37

Figure 10: GA increases the percentage of splenic $CD8^+$ T cells and B cells 38

Figure 11: Effect of Zn^{2+}, xanthohumol and thymol on ceramide formation in DCs from wild type and $ASM^{-/-}$ mice 40

Figure 12: Dose dependence of the effect of Zn^{2+}, xanthohumol and thymol on ceramide production..... 41

Figure 13: Immunohistochemical analysis of nutrient -induced ceramide formation in wild type and $ASM^{-/-}$ DCs 43

Figure 14: Effect of Zn^{2+}, xanthohumol and thymol on DNA fragmentation in DCs from wild type and $ASM^{-/-}$ mice 44
Figure 15: Dose dependence of the effect of Zn^{2+}, xanthohumol and thymol on DNA fragmentation 45
Figure 16: Effect of xanthohumol, thymol on caspase 8 activity..... 46
Figure 17: Effect of xanthohumol and thymol on caspase 3 activity..... 48
Figure 18: Effect of Zn^{2+}, xanthohumol and thymol on cell membrane scrambling 50
Figure 19: Dose dependence of the effect of Zn^{2+}, xanthohumol and thymol on cell membrane scrambling 51
Figure 20: Effect of C2-ceramide on DC apoptosis..... 52
Figure 21: Effect of Zn^{2+} and thymol on Bcl-2 and Bcl-xL protein abundance 53
Figure 22: Effect of xanthohumol on mitochodrial menbrane potential of wild type DCs 54
Figure 23: Effect of xanthohumol and thymol on cytokine secretion in LPS- treated DCs 55
Figure 24: Effect of Zn^{2+} on cytokine secretion by LPS- stimulated DCs..... 57

ABBREVIATIONS

µM	Micromolar
2-ME	2-mercaptoethanol
AIF	Apoptosis inducing factor
ANOVA	Analysis of Variance between groups
APC	Antigen presenting cell
BCR	B cell receptor
DC	Dendritic cell
ERK	Extracellular signal-regulated protein kinase
FBS	Fetal bovine serum
GA	Gum Arabic
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HLA	Human leukocyte antigen
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LPS	Lipopolysaccharides
MAPK	Mitogen-Activated Protein Kinase
mg	Milligram
MHC	Major histocompatibility complex
Min	Minute
ml	Milliliter
mM	Millimolar (mmol/L)
NADPH	Nicotinamide adenine dinucleotide phosphate
NEAA	Non-essential Amino Acid Solution
NF-κB	Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
NK	Natural killer cell
P/S	Penicillin-streptomycin
PBS	Phosphate-Buffered Saline
PS	Phosphatidylserine
RPMI	Roswell Park Memorial Institute
SEM	Standard error

SM	Sphingomyelin
Smase	Sphingomyelinase
TCR	T cell receptor
Th	T heper cell
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-alpha
WBC	White blood cell

ABSTRACT

Dendritic cells (DCs) are antigen-presenting cells involved in the initiation of both innate and adaptive immunity and are thus critically important for the regulation of the immune response to pathogens. Furthermore, they also prevent potentially damaging immune responses being directed against the multitude of harmless antigens, to which the body is exposed daily**.** This role is particularly important in the intestine, where the local immune responses require a tight control, the outcome of which is in the most cases the induction of tolerance. Tolerant immunity connected to DCs can include down-regulation of their maturation, enhanced production of anti-inflamatory cytokines and/or driving cells into apoptosis. In addition, local T cell immunity is an important compartment of the specific intestinal immune system. DCs have the unique ability to activate naïve T cells. They can determine whether non-responsiveness (tolerance) or an active immune response occurs, whether a type 1 or type 2 response predominates. In the intestine, therefore, DCs are required to perform their dual roles very efficiently to protect the body from the dual threats of invading pathogens and unwanted inflammatory reactions.

In the present study it was shown that nutrients, such as thymoquinone, Gum Arabic (GA), zinc (Zn^{2+}), xanthohumol or thymol acted on different aspects of DC functions. Nutrients can stimulate (GA, Zn^{2+}) or decrese (thymoquinone, thymol, xanthohumol) expression of maturation markers and/or cytokine production, influence DC phagocytotic capacity (GA), lead to activation of mitogen-activated protein kinases (MAPK, GA).

Many of the nutrients studied turned out to be strong inducers of DC apoptosis. To determine the signalling pathways involved, DCs from both wild type and gene targeted mice lacking functional acidic sphingomyelinase ($ASM^{-/-}$) were exposed to nutrients and different apoptosis markers assessed. Nutrient (Zn^{2+}, xanthohumol, thymol)-induced apoptosis was triggered by acid sphingomyelinase activation, leading to ceramide formation and subsequent caspase activation, DNA fragmentation and cell membrane scrambling. In addition, regulation of Bcl-2 family members is known to follow ceramide formation, and thus the involvement of Bcl-2 proteins was tested in nutrient-treated DCs. Several nutrients (Zn^{2+}, thymol) were shown to induce down-regulation of anti-apoptotic proteins Bcl-2 and Bcl-xL. The nutrient-triggered cell suicidal death was virtually absent in DCs from $ASM^{-/-}$ mice. In DCs from both genotypes exogenously added C2-ceramide resulted in the induction of cell death, indicating that ceramide production could be a critical step in nutrient-induced DC death.

ZUSAMMENFASSUNG

Dendritische Zellen (DZ) sind Antigen-präsentierende Zellen welche an der Aktivierung angeborener sowie adaptiver Immunität beteiligt sind und deshalb für die Steuerung der Immunantwort in Hinblick auf Pathogene äußerst wichtig sind. Darüber hinaus verhindern sie potentiell schädliche Immunantworten gegen eine Vielzahl von unbedenklichen Antigenen denen der Körper täglich ausgesetzt ist. Diese Aufgabe ist insbesondere im Darm wichtig, wo lokale Immunantworten eine strenge Kontrolle benötigen und in den meisten Fällen zu Induktion von Toleranz führen. Mit DZ verbundene Immuntoleranz kann eine Herunterregelung ihrer Reifung, eine verstärkte Produktion von anti-inflammatorischen Cytokinen und/oder eine Einleitung der Apoptose in den Zellen beinhalten. Darüber hinaus ist die lokale T-Zell-Immunität ein wichtiger Teil des spezifischen intestinalen Immunsystems. DZ haben die einzigartige Eigenschaft naive T-Zellen zu aktivieren. Sie bestimmen ob eine nicht-responsive (Toleranz) bzw. aktive Immunantwort eintritt bzw. eine Typ 1 oder Typ 2 Antwort vorherrscht. Wegen ihrer dualen Rolle werden DZ im Darm benötigt, wo sie den Körper von der zweifachen Bedrohung durch eindringende Pathogene, sowie vor unerwünschten Entzündungsreaktionen schützen.

In der vorliegenden Studie wurde gezeigt, dass Nährstoffe wie Thymoquinon, Gummiarabicum (GA), Zink (Zn^{2+}), Xanthohumol bzw. Thymol auf verschiedene Aspekte der DC-Funktionen wirken. Nährstoffe können die Expression von Differenzierungsmerkmal sowie die Produktion von Zytokinen stimulieren (GA, Zn^{2+}) bzw. vermindern (Thymoquinon, Thymol, Xanthohumol), das phagozytische Leistungsvermögen (GA) beeinflussen, sowie zur Aktivierung von mitogen-aktivierbaren Proteinkinasen (MAPK, GA) führen.

Wie sich herausstellte, sind viele Nährstoffe starke Auslöser von Apoptose in DZ. Um die beteiligten Signalwege zu bestimmen wurden DZ von Wildtyp- bzw. von genmanipulierten Mäusen, welchen eine funktionelle saure (acid) Sphingomyelinase ($ASM^{-/-}$) fehlt, verschiedenen Nährstoffen ausgesetzt und verschiedene Apoptosemarker bestimmt. Eine Nährstoff (Thymoquinon, Zn^{2+}, Xanthohumol, Thymol)-induzierte Apoptose wurde durch Aktivierung der sauren Spingomyelase ausgelöst, was zur Bildung von Ceramid und in weitere Folge zu einer anschließenden Caspaseaktivierung, DNA-Fragmentierung und zur Ummodilierung der Zellmembran führt. Des Weiteren ist bekannt, dass die Regulation von Mitgliedern der Bcl-2 Familie mit der Ceramidbildung einhergeht, weshalb die Beteiligung von Bcl-2 Proteinen in Nährstoff-behandelten DZ getestet wurde. Einige Nährstoffe (Zn^{2+}, thymol) zeigten eine Verminderung der antiapoptotischen Proteine Bcl-2 und Bcl-xL. Ein durch Nährstoffe ausgelöster zellulärer Zelltod war in DZ von $ASM^{-/-}$ Mäusen nahezu nicht nachweisbar. In DZ beider Genotypen führte eine exogene Zugabe von C2-Ceramid zur Induktion des Zelltods, was darauf hinweist, dass die Ceramidproduktion ein kritischer Schritt im Nährstoff-induzierten DZ-Zelltod darstellen könnte.

1. INTRODUCTION

1.1. Adaptive immune system and innate immunity

Immune systems in vertebrates are divided into two basic categories: innate and adaptive immunity.

1.1.1. Innate immune system

The innate immune system comprises white blood cells which are known as leukocytes and mechanisms that defend the host from infection by other organisms, in a non-specific manner. This means that the cells of the innate system recognize and respond to pathogens in a generic way. Innate immune system provides immediate defense against infection, and is found in all classes of plant and animal life (Rasmussen et al 2009).

The major functions of the vertebrate innate immune system include (Janeway, Jr. 2001; Kumar et al 2009; Mogensen 2009)**:**

- Recruiting immune cells to sites of infection and inflammation, through the production of chemical factors, including specialized chemical mediators, called cytokines.
- Activation of the complement cascade to identify bacteria, activate cells and to promote clearance of dead cells or antibody complexes.
- The identification and removal of foreign substances present in organs, tissues, the blood and lymph, by specialized white blood cells.
- Activation of the adaptive immune system through a process known as antigen presentation.

The innate leukocytes include:

- Natural killer cells (NK), Natural killer T cells (NK T) and T cell receptor γδ (TCR-γδ) lymphocytes constitute particular populations of lymphocytes which play important roles in innate immunity and share similar functions upon activation, such as expansion, secretion of soluble factors (cytokines, chemokines) and cytolytic activity (Hamerman et al 2005; Lauwerys et al 2000; Vivier 2006).
- Mast cells: are resident cells of several types of tissues which contain many granules rich in histamine and heparin. Although best known for their role in allergy and anaphylaxis, mast cells play an important protective role as well, being intimately

involved in wound healing and defense against pathogens (Galli et al 2008; Heib et al 2008).

- Eosinophils: are one of the immune system components responsible for combating infection and parasites in vertebrates. Along with mast cells, they also control mechanisms associated with allergy and asthma. They are granulocytes that develop during haematopoiesis in the bone marrow before migrating into blood (Blanchard and Rothenberg 2009; Trivedi and Lloyd 2007).
- Basophils: contain large cytoplasmic granules, have many similar characteristics as mast cells such as: both cell types store histamine. Like all circulating granulocytes, basophils can be recruited out of the blood into a tissue when needed (Schwartz 2002).
- The phagocytic cells including macrophages, neutrophils and dendritic cells: function within the immune system by identifying and eliminating pathogens that might cause infection (Aderem and Underhill 1999).

1.1.2. Adaptive immune system

The adaptive immune system is composed of highly specialized, systemic cells and processes that eliminate or prevent pathogenic challenges. The adaptive immune response provides the vertebrate immune system with the ability to recognize and remember specific pathogens (to generate immunity), and to mount stronger attacks each time the pathogen is encountered. It is called adaptive immunity because the body's immune system prepares itself for future challenges (Janeway, Jr. 2001).

Lymphocytes (B and T) are the essential players in the adaptive immune response. The adaptive immune response takes longer to develop than the innate immune response. Specificity and memory are the distinguishing characteristics of the adaptive immune response. The adaptive immune system can provide a more effective protection against pathogens through their ability to recognize and remember an impressive number of antigens. Memory B and T cells provide the host with the ability to mount much more effective immune responses against secondary infections. Lymphocytes have specific antigen receptors (BCR on B cells and TCR on T cells) created by genetic rearrangements of variable areas during lymphocyte ontogeny. Thus, each naive lymphocyte has an antigen receptor with a unique specificity. They build a repertoire of polyclonal lymphocytes able to respond to a multitude of antigens. B cells contribute to the immune response by secreting antibodies

(humoral immunity), whereas T cells act primarily in cell-mediated immunity. T cells can be subdivided into T helper cells (CD4+, T cells expressing CD4 are known as $CD4^+$ T cells) and T cytotoxic cells (CD8+, T cells expressing CD8 are $CD8^+$ T cells) (Dent and Kaplan 2008; Konig et al 2002).

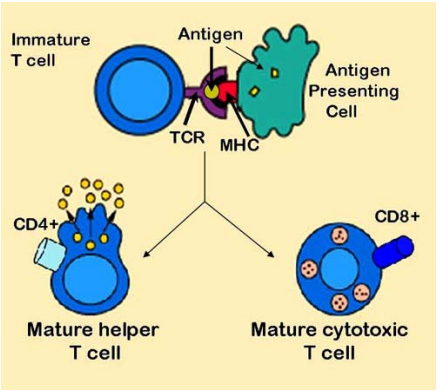

Figure A: Antigen presentation stimulates T cells to become either "cytotoxic" $CD8^+$ cells or "helper" $CD4^+$ cells.

B cells recognize their antigens via their BCR. T cells cannot recognize the antigen without some assistance. The antigenic determinant must be presented by an appropriate major histocompatibility complex (MHC) molecule. Thus, they recognize their antigens through their TCR in the form of an MHC/ peptide complex. CD8+ T cells "see" their antigens in the form of a peptide/MHC class I complex, whereas CD4+ T cells recognize their antigens as a peptide/MHC class II complex. MHC class I molecules are expressed at the surface of all nucleated cells, whereas MHC class II molecules are expressed only by professional antigen-presenting cells (APCs), such as dendritic cells (DCs) (Cruz, Jr. and Bergstresser 1990).

It is important to note that the cells of the innate immune system are critical to the initiation of the adaptive immune response. Thus, APC activation is the first step in the induction of adaptive immunity. DCs generally absorb antigens from the environment, and once they are activated (mostly by microbial compounds), they mature and migrate to the adjacent lymphoid tissue. To be activated, T cells must not only recognize their specific antigen in the form of a peptide/MHC complex, they also need a costimulation signal that is provided by the activated APCs (Joffre et al 2009).

Once activated, naive T lymphocytes proliferate and differentiate into effector cells. CD8+ T cells become cytotoxic (CTL), at which point they can target infected cells. CD4+ T helper (Th) cells control the immune response by activating and regulating other cells such as macrophages and B cells (Williams et al 1991).

1.2. Nature of DCs

DCs are antigen-presenting cells that initiate and modulate the host immune responses by priming T cells (Steinman 271-96;Banchereau et al. 767-811) and controlling the activation of B cells (Banchereau and Steinman 245-52). DCs are responsible for: 1) induction of $CD4^+$ T lymphocyte type 1 and type 2 subset differentiation (Alaniz et al. 3725-35;Maldonado-Lopez and Moser 275-82), 2) $CD8^+$ T lymphocyte activation and enhancement of cytotoxic T lymphocyte activity (Smith et al. 1143-48) and 3) B lymphocyte maturation, Ig class-switching and antibody production (Gerloni, Lo, and Zanetti 516-24;Macpherson, Kushnir, and Wykes 325-34). Their specialized capacities for acquiring, processing, retaining, and finally presenting peptides on major histocompatibility complex (MHC) molecules are critical properties that account in part for their superior role in antigen presentation (Trombetta and Mellman 975-1028).

DCs are generated in the bone marrow and migrate as precursor cells to sites of potential entry of pathogens such as skin, respiratory tract, and lung, where they reside as immature cells in the epithelia of skin and mucosal tissues (Figure. 2). DCs issued from the bone marrow origin express myeloid markers CD13, CD33, and CD11c and have a common progenitor with monocytes/macrophages and granulocytes. They are involved in stimulating naïve T cells. In contrast, lymphoid DCs, which develop from thymic precursors participate in immune tolerance by eliminating auto-reactive T cells.

Following antigen uptake (Figure. 2), immature DCs migrate to lymphoid organs via blood vessels. Immature DCs recognize pathogen-associated molecular patterns (PAMPs) of microbial products via pattern-recognition receptors (PRRs), including the families of Toll-like receptor (TLR) and mannose-like receptors, and inflammatory compounds released by damaged tissues. Upon recognizing a pathogen, DC become mature, a phenotype manifested by their unique capacity to efficiently prime T cells by loss of endocytic and phagocytic receptors; increasing production of proinflammatory cytokines (IL-10, IL-6, IL-12, IL-4 and TNFα); increasing expression of co-stimulatory molecules (CD40, CD80, CD86, MHC class

II, and ICAM-1) (Lane and Brocker 308-13) and acquisition of the responsiveness to homeostatic chemokines, including CCL19 and CCL21 via upregulation of CCR7.

Subsequently, DCs enter the draining lymph nodes in the T-cell-rich zone, present the processed antigens to T lymphocytes in an MHC-restricted fashion (Banchereau and Steinman 245-52;Steinman 271-96) to induce their activation and differentiation into effector cells. After antigen presentation, mature DCs are programmed to undergo apoptosis. Some immature DCs can migrate directly into thymus and lymphoid tissues, where they participate actively in the T cell education/selection and the elimination of auto-reactive T cells, respectively. Activated T cells eliminate microbes, and B lymphocytes mature into plasma cells secreting antibody that neutralizes pathogens (Harizi and Gualde 2005).

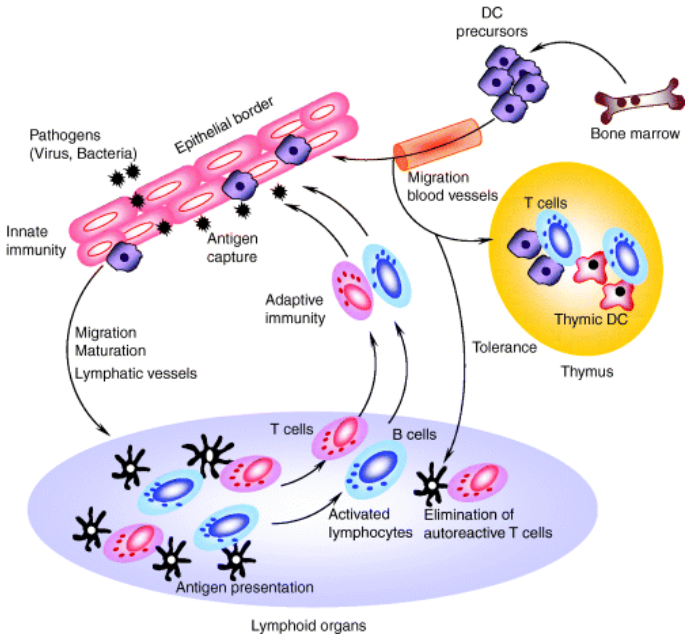

Figure B : DC biology: differentiation, migration, antigen presentation, and tolerance. (Harizi and Gualde 2005)

1.3. Interaction of DCs with NK, B and T cells

DCs are now known to influence many different classes of lymphocytes (B, NK, NKT) and many types of T cell responses (Th1/Th2, regulatory T cells, peripheral T cell deletion) (Lutz and Schuler 2002).

DCs activate naïve Th cells by: 1) the antigen-specific recognition of peptides in the context of MHC molecules on the DC by TCR; 2) co-stimulatory molecules CD80 and CD86 which bind to CD28 on the T cell (Linsley et al 1990) and direct T-cell differentiation into various subsets such as Th1 and Th2 by secreting polarizing cytokines which bind to their receptors on the T cell *(Scott 1993)*. A given Th-cell subset is characterized by its cytokine-secretion profile which is intimately associated to the effector functions. Th1 cells mediate cellular immunity against intracellular bacteria and viruses by secreting cytokines such as IFN-γ and tumour necrosis factor-α (TNF-α), Th2 cells regulate humoral immunity and immunity against extracellular parasites by producing IL-4, IL-5 and IL-13 (Corthay 2006).

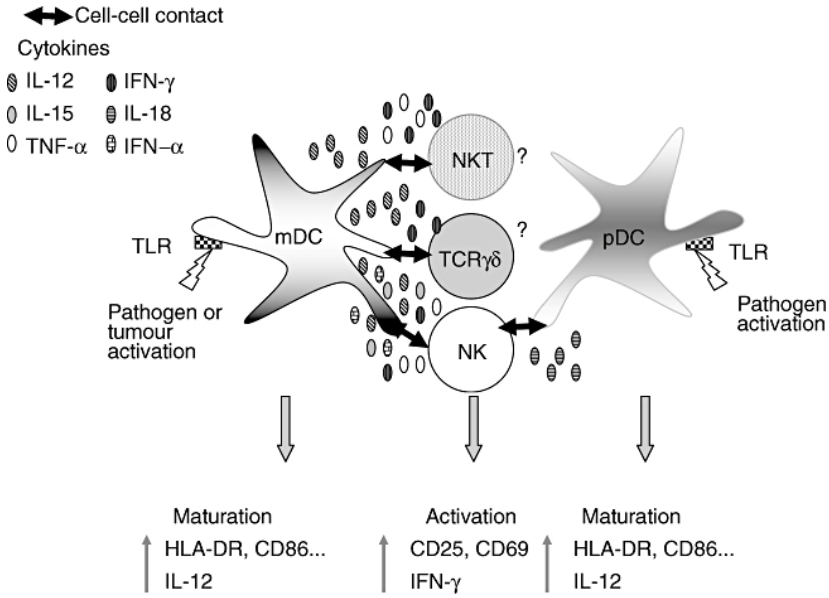

Figure C: Schematic representation of DC-innate lymphocyte cross-talk in the activation of the immune response (Reschner et al 2008b)

The cross-talk between innate cells and DCs which leads to innate lymphocyte activation and DC maturation was found to be multi-directional, involving not only cell–cell contacts but also soluble factors. The final outcome of these cellular interactions may have a dramatic impact on the quality and strength of the down-stream immune responses. In addition to their role in induction of adaptive immune responses, DCs also activate natural killer (NK) cells (Fernandez et al 1999) and can produce large amounts of interferon upon encounter with viral pathogens (Kadowaki et al 2000), thus, providing a link between the adaptive and innate immune system. More recently, DC activating ability was extended to other cell types such as NK T or TCR-γδ cells (Takahashi et al 2002). Moreover, certain DC subsets share common developmental pathways with NK cells, suggesting that these cells could influence each other during differentiation (Marquez et al 1998).

1.4. Tolerance immunity and Th1/Th2 balance drived by DCs

Besides the essential immunostimulatory function of DCs, consolidated findings from the DC research field in the last 10 years have shown that DCs have an additional important function. They act as pivotal players in the peripheral tolerance network by active induction of T cells with immunosuppressive functions and regulation of T effector cell activity (Steinman et al 1997). DCs can determine whether non-responsiveness (tolerance) or an active immune response occurs, whether a type 1 or type 2 response predominates, and they may control tissue specific homing of antigen specific effector cells. Non-responsiveness is not confined to self antigens but can also be induced to foreign antigens if they are delivered in a way that avoids activation of DCs (Hawiger et al 2001a).

Data are now accumulating that mature DCs are potent APCs, but immature DCs have a crucial role in the maintenance of peripheral tolerance to self-antigens (Steinman and Nussenzweig 2002c), inducing regulatory T cells *in vitro* (Sallusto and Lanzavecchia 1999) and *in vivo* (Dhodapkar et al 2001), so that DCs are important mediators of peripheral immune tolerance and maintenance of immune homeostasis (Gad et al 2003).

Regulatory T cells (Treg) are involved in the control of peripheral tolerance (Shevach et al 2001) and the prevention of vigorous inflammatory reactions. Although the exact mechanisms by which Treg exert their suppressive functions are not yet elucidated, two major Treg subsets: a natural population that suppresses through cell-to-cell contact, and an induced population that secretes suppressive cytokines (e.g IL-10) have been demonstrated to play a role.

Interesingly, treatment of immature DCs with IL-10 *in vitro* induces an immunoregulatory phenotype that results in inhibition of $CD4^+$ and $CD8^+$ T lymphocyte reactivity in an antigen-specific manner (Steinbrink et al 1997; Yang and Lattime 2003b). Among $CD4^+$ T lymphocytes, both Th1 and Th2 responses can be inhibited by IL-10 treated DCs (Haase et al 2002b). Similarly, IL-10 treated DCs mediate tolerance within $CD8^+$ cytotoxic T lymphocytes. Moreover, IL-10 pretreatment effectively inhibits maturation of immature DCs. Upon lipopolysaccharide (LPS) stimulation, human and murine IL-10 treated DCs fail to 1) secrete proinflammatory cytokines including IL-1β , IL-6, IL-12p70, and TNF-α, 2) upregulate expression of costimulatory molecules such as CD40, CD80 and CD86 and 3) induce T-lymphocyte proliferation in allogeneic mixed lymphocyte reactions (Haase et al 2002a; Yang and Lattime 2003a).

1.5. Toll-like receptors (TLRs)

TLRs are a class of proteins that play a key role in the innate immune system. They are single membrane-spanning non-catalytic receptors that recognize structurally conserved molecules derived from microbes. Once these microbes have reached physical barriers such as the skin or intestinal tract mucosa, they are recognized by TLRs which activates immune cell responses (Iwasaki and Medzhitov 2004).

To date, 10 members of Toll-like receptors (TLRs) have been identified in human, and 13 in mice, and a series of genetic studies have revealed their respective ligands (Fig. 4) (Takeda and Akira 2005a). For example, LPS of Gram-negative bacteria is recognized by TLR4. TLR2, in concert with TLR1 or TLR6, recognizes various bacterial components, including peptidoglycan, lipopeptide and lipoprotein of Gram-positive bacteria and mycoplasma lipopeptide. In particular, TLR1/2 and TLR2/6 discriminate triacyl lipopeptide and diacyl lipopeptide, respectively. TLR3 recognizes double-stranded RNA (dsRNA) that is produced from many viruses during replication. TLR5 recognizes bacterial flagellin. Mouse TLR11 recognizes yet unknown components of uropathogenic bacteria and a profilin-like molecule of the protozoan parasite *Toxoplasma gondii.* TLR7 recognizes synthetic imidazoquinoline-like molecules, guanosine analogs such as loxoribine, single-stranded RNA (ssRNA) derived from human immunodeficiency virus type I (HIV-1), vesicular stomatitis virus (VSV) and influenza virus, and certain siRNAs. While mouse TLR8, which shows the highest homology to TLR7, is thought to be nonfunctional, human TLR8 mediates the recognition of imidazoquinolines and ssRNA. TLR9 recognizes bacterial and viral CpG DNA motifs and

malaria pigment hemozoin (Iwasaki and Medzhitov 2004) (Coban et al 2005; Hemmi et al 2000; Krug et al 2004).

After recognition of microbial pathogens, TLRs trigger intracellular signaling pathways that result in the induction of inflammatory cytokines, type I interferon (IFN) and chemokines (Figure 4). Moreover, signaling from TLRs induces DC maturation with the upregulation of costimulatory molecules. Importantly, TLRs activate a common signaling pathway that culminates in the induction of inflammatory cytokines such as tumor necrosis factor (TNFα), IL-6, IL-1β and IL-12, as well as alternative pathways that induce appropriate effector responses against different types of pathogens (Akira and Takeda 2004).

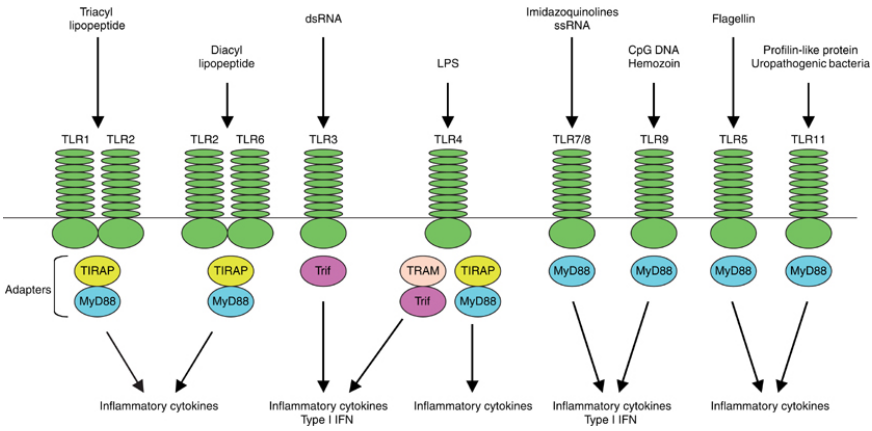

Figure D: TLR-mediated immune responses (Takeda and Akira 2005b)

1.6. Subsets of conventional mouse DCs

One aspect of DC biology that is rapidly evolving is the apparent diversity of DC subsets (Steinman 2007). DCs represent a multi-functional population of cells (Table 1) (Reschner et al 2008a). Conventional DC subsets described in mice include myeloid DCs (mDCs): $CD11c^{+}$ $CD8a^{-}CD11b^{+}$and plasmacytoid DCs (pDCs): $CD11c^{lo}B220^{+}Ly6C^{-}CD11b^{-}$ (Colonna et al 2004; Shortman and Naik 2007a).

Part of these different DC subsets may also be explained by differences in the maturation stage of DCs and the local cytokine environment. The geographical localization of the DC subsets in secondary lymphoid tissues is distinct, myeloid derived DCs mainly migrate to or

reside in the marginal zone (a primary entry point for blood-born antigens), whereas the lymphoid DCs mainly reside in the T-cell areas. This supports distinct functions for the DC subsets, as shown in murine studies (Banchereau and Steinman 1998b; Liu 2001a). It is now well appreciated that the DC subset, its maturation state and the microenvironment or type of pathogen a DC encounters in the periphery, determine the type of immune response that is induced, ranging from a Th1 or Th2 response to immune tolerance (Liu 2001b).

In mice, a small population of mDCs can express CD8 (Shortman and Naik 2007b). These CD8α+ DCs display immuno-regulatory or tolerogenic induction ability (Hawiger et al 2001b; Kronin et al 1996b), but also exhibit the strongest ability to induce a Th1 response (Kronin et al 1996a; Maldonado-Lopez et al 1999) by secreting high amounts of the Th1 polarising cytokine IL-12, in contrast to $CD8^-$ DC (den Haan et al 2000).

Table 1: Populations of DCs in mice and humans.

DC subsets	Tissue distribution	Species	Immature phenotype	Mature phenotype
Bone marrow derived DC	Dermis, airways, intestine, thymus, spleen, liver, lymphoid tissue	Mouse	$CD11c^+CD8\alpha^-$ $CD11b^+$MHC-II^+ TLR-1–$3^{+/-}$TLR-2,4–9^+	$CD83^+$ $CCR7^+$ $CD80^{++}$ $CD86^{++}$ MHC-II^{++}
		Human	$CD1a^+$ $CD14^-$ $CD11c^{++}CD11b^{++}$ $CD1c^+$ $CD209^+$ MHC-II^+ TLR-1, 6^+,3,8^{++}	$CD40^+$
Plasmacytoid DC	Lymphoid organs, liver, lung, skin	Mouse	$CD11c^{+/-}B220^+Ly^-6C^+CD11b^-$ $PDCA^+$ MHC-II^+ TLR-2–9^+	
		Human	$CD14^-CD11c^-CD123^{++}$ $BDCA2^+$ $ILT7^+$ MHC-II^+ TLR-$7,9^{++}$	
$CD8\alpha^+$DC	Thymus, spleen, lymph node, liver	Mouse	$CD8\alpha^+CD4^-CD11c^{++}$ CD11b $CD205^{++-}$TLR-2–4,6,8,9^+	
		Human	Not identified	
Langerhans cells	Mucosal epithelia, epidermis	Mouse	$CD8\alpha^-$ $CD11c^+$ $CD205^{++}$ E-cadherin$^+$$CD207^+$	E-cadherin $^{+/-}$
		Human	$CD14^{+/-}CD11c^+CD1a^+$ E-cadherin$^+$$CD207^+CCR6^+$	
DC, dendritic cells. $^{+/-}$, low; $^{++}$, high.				

1.7. Migration and Phagocytosis

DCs have been shown to phagocytose a variety of materials, including microorganisms and latex beads (Reis e Sousa et al 1993a). The ability of DCs to phagocytose and process antigen is highly dependent upon the stage of DC differentiation (Reis e Sousa et al 1993b). Immature cells residing in the tissue are highly phagocytic and are less efficient at stimulating T lymphocytes via both receptor- and non-receptor-mediated mechanisms. DCs degrade antigens in endocytic vesicles to produce antigenic peptides capable of binding MHC. In response to danger signals, i.e. tissue damage, pathogen-derived products, or inflammatory products produced by neighboring tissue cells, DCs cease their endocytic activity (Sallusto et al 1995), increase their expression of MHC class II molecules (Cella et al 1997), and produce high levels of chemokines (Sallusto et al 1999). These considerable changes, collectively called maturation, occur while DCs migrate to lymphoid organs where they interact with B cells and antigen-specific CD4 T cells to initiate immune responses (Wallet et al 2005).

DCs are highly mobile cells. After antigen capture, DCs migrate to T cell region of draining lymph nodes. DC migration is crucial for the initiation of immune responses and the link of innate immunity to adaptive responses. Microbes and pathogens affect DC migration, recruitment to tissues and their differentiation. Chemokines and chemokine receptors including CCL19 and CCL21 regulate DC trafficking and are under the control of cytokines produced by APCs and neighboring tissue cells (Randolph et al 2008).

1.8. The mucosal immune system

Most of our encounters with antigens or infectious agents occur at mucosal surfaces, which include the surface lining the gastrointestinal, respiratory and genitourinary tracts (Delves and Roitt 2000). Since nutrients are usually absorbed orally, they are thus ideally suited to influence the immune response at the “mucosal frontier” of the gastrointestinal tract, representing more than 300 m^2. Well known for its nutrition function (digestion of food and the assimilation of the nutrients), the intestinal system is also able to protect us from the pathogenic microbes. It contains more than 100 million neurons, secretes at least 20 neurotransmitters identical to those produced by the brain (serotonin, noradrenalin, dopamine, etc.), produces 70 to 85 % of the immune cells of the organism, lodges 100 000 billion bacteria. All these compounds, present locally, are in relationship to the whole of the organism (Delcenserie et al 2008).

Although the immune response of the intestinal mucosa exhibits several features in common with the immune responses produced by other organs, it is characterized by certain distinctive

properties. The immune properties of the digestive mucosa are provided by the GALT (Gut-associated lymphoid tissue). The GALT is composed of lymphoid aggregates, including the Peyer's patches (located mainly in the small intestinal distal ileum), where induction of immune responses occurs, and mesenteric lymphoid nodes. In addition, there are large amounts of immune-competent cells in the lamina propria and the mucosal epithelium (Delcenserie et al 2008).

The intestine also protects us from pathogens because its epithelium is covered by mucus and avoids any direct contact with the microorganisms.The intestinal immune system must encounter all antigens in order to determine which ones require an immune response and which ones can be safely tolerated (Delcenserie et al 2008).

The intestinal immune system is the subject of complex regulation processes allowing the elimination of pathogenic microorganisms, while maintaining a tolerance towards food antigens and endogenous flora. Butyrate as well as other products resulting from colic fermentation, could take part in this regulation (Marteau et al 2004).

1.9. Mucosal dendritic cells

Mucosal DCs are assumed to play key roles in regulating immune responses in the antigen-rich gastrointestinal environment. It has been shown that DCs, using their dendrites, act as guard cells in the intestinal lumen without disturbing the integrity of their tight surface junctions (Niess 2008). Mucosal DCs are a heterogeneous population that can either initiate (innate and adaptive) immune responses, or control intestinal inflammation and maintain tolerance (Nagler-Anderson 2001; Steinman and Nussenzweig 2002b).

The intestinal innate and adaptive immune system has evolved in response to potent stimuli derived from constituents of the commensal microflora. In most cases these local immune responses achieve tolerance to the intestinal microflora and food antigens. Tolerance to intestinal self antigens, oral antigens and the commensal flora is achieved by interactions of DCs with regulatory and effector T cells. Local T cell immunity is an important compartment of the specific intestinal immune system. T cell reactivity is programmed during the initial stage of its activation by DCs (Medzhitov and Janeway, Jr. 1999).

DCs reside in mucosal tissues or recirculate in the blood and lymphoid tissues (Iwasaki 2007). The lamina propria of the small and large intestine are effector sites of mucosal tissues. The local microenvironment influences the phenotype of DCs, and are characterized by a remarkable plasticity between DCs (Kelsall and Rescigno 2004). In the lamina propria of the small and large intestine, DCs are ideally situated to survey the constituents of the commensal microflora and monitor food antigens (Bjorck 2001).

Defects in this regulation are supposed to lead to the several forms of inflammatory disease such as: Crohn's disease (CD) and ulcerative colitis (UC) (MacDonald and Monteleone 2005)

and hemorrhagic rectocolitis (HRC), a deregulation of the intestinal immune system would lead to an inadequate response against one or more endoluminal antigens. An imbalance between Th1 (IL-2, IFNγ, T N F α) and Th2 responses (IL-4, IL-5, IL-10) was described in human and also in animal models (Zeitz et al 1990). This led to a chronic inflammatory answer characterized by the production of pro-inflammatory cytokines (IL-1, IL-6, TNFα). Thus, the cytokine profile plays an important role in the maintenance of intestinal immune homeostasis (Niess 2008).

1.10. Cell death

The mammalian immune system discriminates between modes of cell death: necrosis often results in inflammation and adaptive immunity, whereas apoptosis tends to be anti-inflammatory and promote immune tolerance (Matsue and Takashima 1999).
Apoptosis is characterized by plasma-membrane shrinkage, nuclear condensation, and nucleosomal DNA fragmentation (Matsue and Takashima 1999; Packham et al 1996).

There are two canonical apoptosis pathways: intrinsic and extrinsic (Reuter et al 2008).

1.10.1. Intrinsic apoptosis pathway

It requires mitochondrial-specific signaling. Mitochondria initiate apoptosis through mitochondrial outer membrane permeabilization and the release of apoptogenic factors (*e.g* cytochrome c, AIF- apoptosis inducing factor) from the mitochondrial intermembrane space, leading to cell death through caspase-dependent and –independent pathways (Green and Reed 1998a; Mohamad et al 2005).
Caspases are known to mediate some of the apoptotic features, such as DNA fragmentation, chromatin condensation, membrane blebbing, cell shrinkage, and formation of membrane-enclosed vesicles (apoptotic bodies). Signaling cascades can also affect the inner mitochondrial membrane permeability in apoptosis and necrosis. As a consequence, cells also exhibit a loss of electrical potential across the inner membrane which is quantifiable by means of potentiometric dyes (Marchetti et al 1996).
Apoptosis is also regulated by caspase-independent mechanisms. For example, apoptosis has been induced by disrupting the mitochondrial function in the absence of caspase activities in nucleated cells (Green and Reed 1998b). Mitochondrial dysfunction, characterized by marked reduction in mitochondrial membrane potential ($\Delta\psi m$), is an early step of ongoing DC death that can be triggered by many cytotoxic stimuli (McLellan et al 2000; Nencioni et al 2006b; Vassiliou et al 2004a). The participation of mitochondria in the regulation and amplification of the apoptotic cascade is regulated by proteins of the Bcl-2 family. Compelling evidence

indicates that mitochondria-related proteins of the Bcl-2 family are crucial DC death sensors (Nencioni et al 2006a; Nicolo et al 2001; Vassiliou et al 2004b), substantiating the importance of mitochondria in DC apoptosis.

1.10.2. Extrinsic apoptosis pathway

It is mediated by death receptors, such as the receptors for Fas and tumor necrosis factor (TNF)- related apoptosis-inducing ligand (TRAIL), and caspase-8, i.e. the major initiator caspase in this pathway. Several death receptors, including Fas, are expressed in DCs. However, DCs are known to be resistant to Fas-induced cell death through the constitutive expression of FLICE–like inhibitory protein (FLIP), a strong inhibitor of apoptosis initiated by death receptors (Willems et al 2000)

1.10.3. Sphingomyelinase pathway

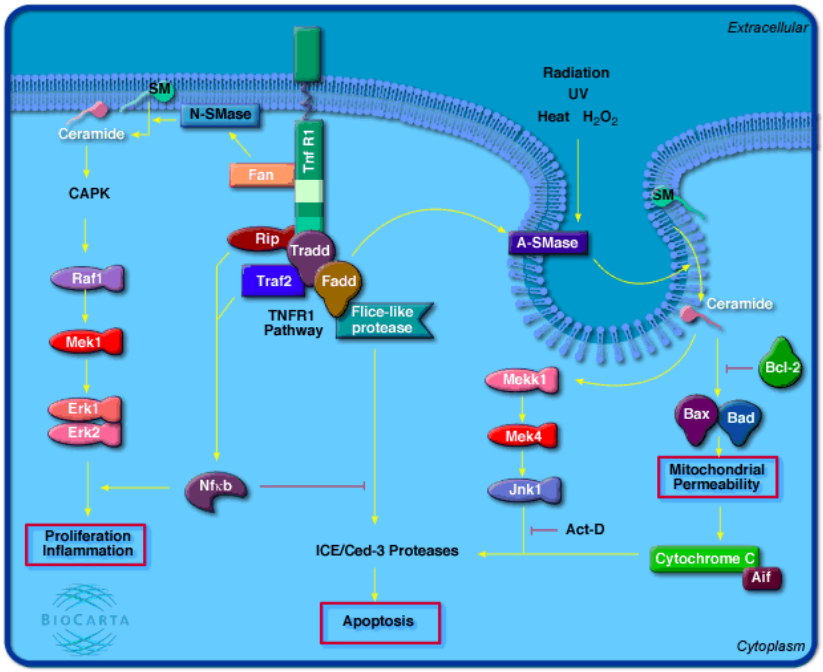

Figure E: Signalling through sphingomyelinase pathway

The sphingomyelin pathway is a signal transduction system initiated by hydrolysis of plasma membrane phospholipid sphingomyelin to ceramide via a sphingomyelinase. Sphingophospholipid sphingomyelin (SM) is distributed almost throughout all subcellular membranes, although it is concentrated in the outer leaflet of the plasma membrane (Koval and Pagano 1991) and provides a barrier to the extracellular environment (Kolesnick 1991). Sphingomyelin degradation is catalyzed by sphingomyelinase (SMase) to phosphocholine and ceramide (*N*-acylsphingosine) upon cell stimulation (Pettus et al 2002).

Two major SMases enzymes are known to induce ceramide formation: neutral SMase (NSMase) and acid SMase (ASMase). They are rapidly activated by diverse stress stimuli and promote an increase in cellular ceramide levels over a period of minutes to hours (Levade and Jaffrezou 1999). NSmase (pH optimum 7.4) was logically suggested to function at the plasma membrane (Dressler et al 1991; Haimovitz-Friedman et al 1994) and ASMase was originally considered to be a strictly lysosomal enzyme because of its pH optimum at 4.5–5.0 (Schutze et al 1992).

1.10.4. Regulation of apoptosis by ceramide

Ceramide, a product of sphingolipid metabolism, is a second messenger involved in regulating numerous cellular functions, including proliferation, differentiation, apoptosis, and cytokine release (Ballou et al 1996; Hannun and Obeid 1995). Ceramide is generated in response to various stress stimuli like death receptors (such as CD95, DR5, or TNF) triggering. Second, nonreceptor stimuli mediating cell death, such as irradiation, heat shock, cytotoxic drugs, H_2O_2, toxins, UV light, bacteria, and viruses can lead to ceramide formation. Finally, third, ceramide can be formed when the cell death is induced upon growth factor deprivation or the disruption of the cell's contact with its matrix (Carpinteiro et al 2008b; Grassme et al 2008a; Jana et al 2009b; Lang et al 2008b; Perrotta et al 2008a; Smith and Schuchman 2008a).

Ceramide may also be converted back to SM by transfer of phosphorylcholine from phosphatidylcholine to ceramide via SM synthase (Hannun et al 2001).

1.10.5. The Bcl-2 family: inhibitors and promoters of apoptosis

The fate of a cell following an apoptotic stimulus may be determined by the balance of expression of various Bcl-2 family members, i.e., the pro- vs. the antiapoptotic members, and the degree of caspase activation attainable (Korsmeyer 1995). The expression of various Bcl-2 members is regulated in a cell type- and stimulus-dependent manner. Survival signals up-regulate the expression of Bcl-2 or Bcl-x whereas death signals downregulate their expression

and may concomitantly up-regulate Bax, Bad or other proapoptotic members (Chen et al 1995). In mammalian systems, Bcl-2 and Bcl-x_L are the main members of this family that inhibit apoptosis. These proteins are located predominantly at the outer mitochondrial membrane, endoplasmic reticulum, and nuclear membrane (Szegezdi et al 2009).

The function of some Bcl-2 family members is also regulated at the post-translational level. Bcl-2 can be phosphorylated on serine residues which may either enhance or inhibit its antiapoptotic function (Haldar et al 1994; Haldar et al 1995). Bad, a proapoptotic member, is inactivated by phosphorylation in response to survival signals (Zha et al 1996). In the dephosphorylated state, Bad preferentially binds to and inhibits Bcl-2 function, thus promoting apoptosis (Murphy et al 2005).

The interactions between the antiapoptotic members, *e.g.*, Bcl-2 and Bcl-xL, with the proapoptotic members, *e.g.*, Bax, Bak, and Bad have been demonstrated to be critical in determining the fate of the cell (Sedlak et al 1995; Yang et al 1995).

Additionally, Bcl-2 was recently shown to be cleaved by caspase-3 in its loop domain to yield a fragment that is functionally and structurally similar to Bax and which further promotes the apoptotic cascade (Cheng et al 1997).

1.10.6. Caspase activity

Caspases, a unique family of cysteine-dependent aspartate specific proteases, play a pivotal role in cell death. The mammalian genome encodes fourteen distinct caspases, seven of which were shown to function in apoptosis (Chowdhury et al 2008).

Initiator caspases are activated through dimerization facilitated at multi-protein complexes. Activation of caspase-9, the initiator caspase of the intrinsic pathway, while the apical caspase of the extrinsic apoptotic pathway caspase-8 is activated within the death-inducing signaling complex (DISC) (Ashkenazi and Dixit 1998; Boatright and Salvesen 2003b). On the other hand, activation of effector caspases, such as caspase-3 and -7 occurs upon their cleavage at specific internal aspartic acid residues by initiator caspases (Boatright and Salvesen 2003a). Downstream of this activational cascade, caspases cleave a variety of regulatory and structural proteins and important enzymes, ultimately leading to cell death (Gradzka 2006; Pop and Salvesen 2009).

1.11. Nutrients

The immune system acts to ensure tolerance to ‘self’, to food and other environmental components, and to commensal bacteria. The development of tolerance is the result of active

immune mechanisms requiring antigen-contact and acting in a T cell-dependent fashion (Faria and Weiner 2005; Powell 2006; Taylor et al 2006). Nutrition may be the source of antigens to which the immune system must become tolerant, provide factors, including nutrients, that themselves might modulate immune maturation and responses.

In the intestine, a key requirement of DCs is to generate oral tolerance to food antigens, prevent potentially harmful immune responses against nonpathogenic antigens while preserving immunity to pathogens (Cerovic et al 2009; Rescigno et al 1998). DCs have access to the intestinal lumen and are thus exposed to the relatively high concentrations of nutrients in this body compartment (Edelman and Kasper 2008) resulting in the induction of 'non-inflammatory' DC that released IL-10 and IL-6, but not IL-12, and promoted the polarization of T cells toward a Th2 phenotype (Rescigno et al 2001). An inappropriate immune response to microbial antigens of commensal microorganisms in genetically susceptible individuals may lead to chronic inflammatory diseases, such as Crohn's disease or ulcerative colitis (Silva 2009) and the deranged function of the immune system in autoimmune disease (Rescigno et al 2008).

1.11.1. Thymoquinone

Thymoquinone, a nutrient derived from *Nigella sativa* (Badary et al 2007; Khader et al 2009) exerts remarkable anticarcinogenic(Aggarwal et al 2008a; Ali and Blunden 2003b; Gali-Muhtasib et al 2006; Gali-Muhtasib et al 2008c; Mohamed et al 2003; Salem 2005a) and anti-inflammatory (El Gazzar et al 2006a; El Gazzar et al 2006c; El Gazzar et al 2007; El Gazzar 2007; El Mezayen et al 2006; Salem 2005c; Sethi et al 2008b; Tekeoglu et al 2007) potency (Aggarwal et al 2008b; Ali and Blunden 2003a; Gali-Muhtasib, Roessner, and Schneider-Stock 2006; Gali-Muhtasib et al 2008d; Salem 2005b). The anticarcinogenic effect is at least partially due to stimulation of tumor cell apoptosis (Gali-Muhtasib et al 2004b; Roepke et al 2007c; Rooney and Ryan 2005a; Shoieb et al 2003a). Signaling involved in the proapoptotic effects of thymoquinone includes p53-(Gali-Muhtasib et al 2004a; Gali-Muhtasib et al 2008b; Roepke et al 2007b) and NFκB- (Sethi et al 2008a) dependent gene expression, suppression of AKT and extracellular signal-regulated kinase (Yi et al 2008), decrease of reduced glutathion (Rooney and Ryan 2005b) and caspase activation (El Mahdy et al 2005; Rooney and Ryan 2005c). Moreover, thymoquinone influences the formation of prostaglandins and leukotrienes (El Gazzar et al 2006b).

1.11.2. Gum Arabic

Gum Arabic (GA) is a water-soluble (Tiss et al 2001) polysaccharide based on branched chains of (1-3) linked β-D-galactopyranosyl units containing α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl and 4-O-methyl-β-D-glucuronopyranosyl units (Deckwer et al 2006). It is fabricated from gummy exudates of *Acacia Senegal* (Younes et al 1995). In the colon GA is fermented by microorganisms to short chain fatty acids {Phillips, 1998 21 /id}. Its is considered one of the safest dietary fibers (Anderson 1986).

In Middle Eastern countries GA is used in the treatment of patients with chronic kidney disease and end-stage-renal-disease (Al Majed et al 2002b). GA increases fecal nitrogen excretion {Bliss, 1996 46 /id} and decreases production of free oxygen radicals (Al Majed et al 2002a). Intestinal GA fermentation leads to the formation of several degradation products including short-chain fatty acids (Bliss 2004a). Accordingly, GA treatment may enhance serum butyrate concentrations (Matsumoto et al 2006a).

In the intestine, GA may modify the function of the reabsorbing epithelial cells (Nasir et al 2008b). At least in theory, GA may modify the function of intestinal DCs.

1.11.3. Zn^{2+}

Zinc (Zn^{2+}) is the second most prevalent trace element in the body, an essential structural component of a great number of proteins, including enzymes and transcription factors. It can act as a second messenger and neurotransmitter and is important for cell replication, tissue repair and growth (Murakami and Hirano 2008a). Zn^{2+} is an essential nutrient (Prasad 1995), which counteracts a variety of infectious diseases (Cuevas and Koyanagi 2005; Fischer-Walker and Black 2004) including malaria (Richard et al 2006c), diarrhea (Long et al 2006; Richard et al 2006b), and respiratory infections (Coles et al 2007; Hambidge 2006; Richard et al 2006a). Zn^{2+} supplementation is considered particularly important in infants and children (Brown et al 2007; Georgieff 2007; Krebs and Hambidge 2007; Sheng et al 2006), pregnant women (Litonjua et al 2006) and elderly (Prasad et al 2007) and has been proven beneficial in burns (Berger and Shenkin 2007). Zn^{2+} deficiency has been shown to predispose to bacterial and viral infection (Fischer-Walker and Black 2004; Fraker and King 2004; Prasad 1998a). Zn^{2+} depletion and Zn^{2+} supplementation influence functions of both innate and adaptive immunity (Hosea et al 2003b; Prasad 2000a); Ibs and Rink 2003b).

Zn^{2+} depletion by the chelating agent TPEN (N,N,N´,N´-tetrakis (2-pyridylmethyl ethylendiamine) has been shown to upregulate DC surface expression of MHCII and CD86 and to enhance $CD4^+$ T cell stimulatory activity, effects reversed by addition of Zn^{2+} (Kitamura et al 2006d). On the other hand, LPS stimulation of DCs has led to a decrease in

intracellular free Zn^{2+} via alterations in Zn^{2+} transporter expression and this decrease is involved in DC maturation (Kitamura et al 2006c). Little is known, however, about the signaling involved. In other cell types, Zn^{2+} has been shown to activate a secretory sphingomyelinase leading to the formation of ceramide (Tabas 1999b).

1.11.4. Xanthohumol

Xanthohumol, a flavonoid from beer (Intelmann et al 2009; Keukeleire et al 2007; Kodama et al 2007), has been shown to elicit anti-inflammatory, antiangiogenic, anticancer, antibacterial, antifungal, antimalarial and antiviral effects (Cho et al 2008a). It may further favourably influence sleep disorders and menopausal symptoms in women (Guo et al 2006). The anticarcinogenic effect is thought to result from inhibition of cell proliferation and stimulation of apoptosis (Colgate et al 2007c; Dell'Eva et al 2007b; Diller et al 2005b; Gerhauser et al 2002a; Gerhauser 2005; Goto et al 2005c; Guerreiro et al 2007a; Harikumar et al 2009d; Ho et al 2008a; Larghero et al 2007a; Lust et al 2005a; Monteghirfo et al 2008b; Monteiro et al 2007a; Monteiro et al 2008a; Pan et al 2005c; Vanhoecke et al 2005c). The effect of xanthohumol on the immune response has been attributed to an influence on the function of lymphocytes (Choi et al 2009a; Harikumar et al 2009c; Wang et al 2004) or macrophages (Cho et al 2008b).

In lymphocytes, immunosuppressive effects of xanthohumol were reported which include inhibition of T cell proliferation, cell-mediated cytotoxicity and Th1 cytokine (IL-2, IFN-gamma and TNF-alpha) production, effects attributed to suppression of NF-kappaB (Gao et al 2009). In contrast, xanthohumol treatment of mouse EL-4 T cells activated with phorbol 12-myristate 13-acetate plus ionomycin, significantly increased IL-2 production through the enhancement of IL-2 promoter, NF-AT and AP-1 activity with no effect on NF-kappaB activity (Choi et al 2009b). In LPS- and IFN-γ- stimulated mouse macrophage RAW 264.7 cells xanthohumol inhibited the production of NO by suppressing the expression of inducible NO synthase (iNOS) (Zhao et al 2003). In LPS-activated RAW264.7 cells xanthohumol was also shown to reduce the expression of the LPS receptor components such as TLR4 and MD2 resulting in the suppression of NF-kappaB activation (Cho et al 2008c). In the IFN-gamma-stimulated RAW264.7 cells, the binding activity of STAT-1alpha and IRF-1 was inhibited by xanthohumol (Cho et al 2008d).

1.11.5. Thymol

Thymol is well known for its antimicrobial (Burt 2004a; Cervenka et al 2008b; Corbo et al 2008a; Lee and Jin 2008b; Razzaghi-Abyaneh et al 2008b)and antifungal (Guo et al 2009)

potency. Little is known, however, about its effect on the host immune system. It has been shown to influence the generation of reactive oxygen species (Kim and Lee 2004; Szentandrassy et al 2004) and, at higher concentrations, to induced DNA damage (Aydin et al 2005; Undeger et al 2009b) and inhibit cell proliferation (Stammati et al 1999). In erythrocytes it protects against suicidal cell death (Mahmud et al 2009).

2. AIMS OF THE STUDY

Nutrition may be the source of antigens to which the immune system must become tolerant in intestinal lumen. They may bind to DCs, which can protrude between epithelial cells and sense the composition of the lumen, and induce DC response. Impaired maturation of DCs as well as enhanced anti-inflamatory cytokine secretion by DCs may drive immunity tolerogenic. In addition, induction of DC apoptosis is expected to weaken the immune response.

The present study explored, whether the following nutrients: thymoquinone, GA, Zn^{2+}, xanthohumol and thymol affect DC functions. Specifically, the following questions were addressed:

1. How do nutrients influence DC maturation and cytokine secretion?
2. How is DC survival affected by nutrients?
3. Which signaling pathways are involved in DC apoptosis induced by nutrients?

A special focus was made on acid sphingomyelinase/ceramide signaling pathway.

3. MATERIAL AND METHODS

3.1. Equipment

- FACS calibur [Becton Dickinson, Heidelberg]
- EXCITER Confocal Laser Scanning Microscope [Carl Zeiss MicroImaging GmbH, Germany]
- Incubator [Heraeus, Hanau]
- PH meter 761 Calimatic [Knick, Berlin]
- Micrometer [Oditest, Kroeplin]
- Cell strainer [BD falcon, Heidelberg]
- Electronic hematology particle counter [Medical Diagnostics Marx, Butzbach]
- QuadroMACS Separator, magnetic separation [Miltenyi Biotec, Bergisch Gladbach]

- Suspension culture plate 96w, 24w, F-bottom, with lid, sterile [Greiner bio-one, Frickenhausen]
- FACS tubes, 1,3ml, PP, round bottom [Greiner bio-one, Frickenhausen]
- Sterile filters [Millipore, Cork, Ireland]
- Syringes, Omnifix-H, 1ml [B Braun, Melsungen]

3.2. Mice

- C57 BL/6N mice (Charles Rivers, Sulzfeld, Germany)
- Acid sphingomyelinase $ASM^{-/-}$ and wild type mice (7-12 week old mice, a kind gift of Dr. Verena Jendrossek (University of Tübingen, Germany), originally obtained from Dr. R. Kolesnick (Sloan Kettering Cancer Memorial Center, NY, USA).)

3.3. Chemicals

- BD FACS Flow solution, BD FACS clean solution, BD FACS Rinse solution [Becton Dickinson, Heidelberg]
- PBS (Phosphate buffer saline, tablets) [GIBCO, Karlsruhe]
- FBS research grade EU- approved (fetal bovine serum) [Hyclone, Perbio, Bonn]
- FACS buffer (1% FBS in PBS solution)
- Annexin washing buffer (125mM NaCl, 10mM Hepes, 5mM $CaCl_2$, pH 7.4) [GIBCO, Karlsruhe]
- Steril medium: RPMI 1640 [GIBCO, Carlsbad, Germany] containing: 10 % FCS, 1 % penicillin/streptomycin, 1 % glutamine, 1 % non-essential amino acids (NEAA) and 0.05 % β-mercaptoethanol
- T cell isolation buffer (degassed): PBS, pH 7.2, supplemented with 0.5% bovine serum albumin and 2 mM EDTA. The buffer was degassed by applying vacuum or sonification.
- Lipopolysaccharide [from *Escherichia coli* Sigma-Aldrich, Germany]
- GM-CSF [Preprotech, Tebu-bio, Rocky Hill, NJ]
- Thymoquinone, thymol, xanthohumol, zinc chloride [Sigma-Aldrich, Germany]
- FITC-conjugated anti-mouse CD11c, PE-conjugated anti-mouse CD86, PE-conjugated rat anti-mouse I-A/I-E, PE-conjugated anti-mouse CD40, PE- conjugated anti mouse ICAM-1 (CD-54), anti-CD16/CD32, FITC-conjugated anti-mouse CD45, PE-conjugated anti-mouse CD19, FITC - labelled goat anti-mouse IgG antibody, FITC-conjugated anti-mouse CD3e, PE-conjugated anti-mouse CD8 [BD Pharmingen, Heidelberg, Germany]

- Anti-ceramide antibodies [Mouse IgM, Alexis, Lörrach, Germany]
- IL-6, IL-10, IL-12p70 and TNFα ELISA kits [BD PharMingen, Heidelberg, Germany]
- FITC-conjugated dextran [Sigma-Aldrich, Taufkirchen, Germany]
- Anti-phospho (p)-ERK, anti-ERK, anti-p-SAPK/JNK, anti-SAPK/JNK, α/ß- Tubulin [Cell Signaling, Danvers]
- Anti p-p38, anti-p38, anti-Bcl-2, anti-Bcl-xL [Santa Cruz, Heidelberg, Germany]
- Enhanced chemiluminescence (ECL) kit [Amersham, Freiburg, Germany]
- Mouse Pan T isolation kits [Miltenyi Biotec, Bergisch Gladbach, Germany]
- Annexin V fluos [Roche Diagnostic, Mannheim, Germany]
- Propidium iodide (PI) [Sigma Aldrich, Taufkirchen, Germany]
- Lysis buffer [Pierce, Rockford, USA]
- Protease inhibitor cocktail [Sigma-Aldrich, Taufkirchen, Germany]
- TBS (Tris-Buffered Saline) [GIBCO, Karlsruhe]
- Tween-20 [Sigma-Aldrich, Taufkirchen, Germany]
- Formaldehyde solution [Sigma-Aldrich, Taufkirchen, Germany]
- RNase A [Qiagen, Germany]
- Caspase 8 and Caspase 3 kits [Biovision, California, USA]
- Tetramethyl-rhodamine ethyl ester (TMRE, Molecular Probes, Leiden, Netherlands)

3.4. DC culturing

DCs were isolated from femurs and tibias of 8-12 weeks old mice as described by {Labeur, 1999 2 /id}. Cells were then plated at a density of $2*10^6$cells/10ml in 60-mm dish and cultured for 7 days in RPMI 1640 (GIBCO, Carlsbad, Germany) containing: 10 % FCS, 1 % penicillin/streptomycin, 1 % glutamine, 1 % non-essential amino acids (NEAA) and 0.05 % β-mercaptoethanol. Cultures were supplemented with GM-CSF (35 ng/mL, Preprotech, Tebu-bio, Rocky Hill, NJ). Cultures were fed with fresh medium containing GM-CSF every 3 days. Nonadherent and loosely adherent cells were harvested after 7-8 days of culture. Most (80% or more) of the cells expressed CD11c, which is a marker for mouse DCs. DCs generated only in the presence of GM-CSF display an immature phenotype. To obtain mature DCs, LPS was added to the culture medium. Experiments were performed on DCs at days 7-10. DCs ($5*10^5$cells/3ml) were then treated with the following nutrients:

- Gum Arabic (0.5%, 24h).

- LPS (from *Escherichia coli*, Sigma-Aldrich, Germany, 200 ng/ml, 24 h) in the absence or in the presence of different concentrations of thymoquinone (1-20 µM, Sigma-Aldrich, Germany)
- Zn^{2+} (10-1000 µM, 24h, Sigma-Aldrich, Germany)
- Thymol (2-100 µg/ml, 24h, Sigma-Aldrich, Germany)
- Xanthohumol (2-50 µM, 24h, Sigma-Aldrich, Germany)

3.5. Immunostaining and flow cytometry

Surface expression of CD11c, CD86, MHC class II, CD54 and CD40 was determined by FACS analysis. To this end, cells (4 x 10^5) were incubated in 100 µl FACS buffer (PBS plus 0.1% FCS) containing fluorochrome-conjugated antibodies at a concentration of 10 µg/ml. A total of 2 x 10^4 cells were analyzed. The following antibodies (all from BD Pharmingen, Heidelberg, Germany) were used for staining: FITC-conjugated anti-mouse CD11c, clone HL3 (Armenian Hamster IgG_1, λ2), PE-conjugated anti-mouse CD86, clone GL1 (Rat IgG_{2a}, κ), PE-conjugated rat anti-mouse I-A/I-E, clone M5/114.15.2 (IgG2b, κ), PE-conjugated anti-mouse CD40 clone 3E2 (Rat IgG_{2a}, κ) and PE- conjugated anti mouse ICAM-1 (CD-54), clone 3E2 (Armenian Hamster IgG1, κ). After incubating with the antibodies for 60 min at 4^0C, the cells were washed twice and resuspended in FACS buffer for flow cytometry analysis.

3.6. Cytokine quantification in cell supernatants

DCs (1.66*10^5cells/ml) were treated for 24h with LPS (200 ng/ml) in the absence or in the presence of different concentrations of either thymoquinone (1-20 µM) or Zn^{2+} (10-1000 µM) or thymol (2-100 µg/ml) or xanthohumol (2-50 µM). Alternatively, DCs were treated for 24 h with Gum Arabic (0.5%).

Cell culture supernatant was collected and stored at -20˚C until use for ELISA. For analysis of IL-6, IL-10, IL-12p70 and TNFα concentrations, commercially available ELISA kits (BD PharMingen, Heidelberg, Germany) were used according to the manufacturer's protocol.

3.7. DC phagocytosis assay

DCs (1.66*10^5 cells/ml) were treated with either LPS (200 ng/ml, 24 h) or Gum Arabic (0.5%, 24h). Cells were centrifuged at 1500 rpm, 5 min, then suspended in prewarmed serum-free RPMI 1640 medium, pulsed with FITC-conjugated dextran (Sigma-Aldrich, Taufkirchen,

Germany) at a final concentration of 1 mg/ml and incubated for 3h at 37^0C. Uptake was stopped by adding ice-cold PBS. Then the cells were washed three times with ice cold PBS supplemented with 5% FCS and 0.01% sodium azide before FACS analysis. DCs were analyzed for the uptake of FITC-dextran.

3.8. Immunoblotting

DCs ($1.66*10^5$ cells/ml) were either unpulsed (control) or pulsed with:

- LPS or Gum Arabic for 10, 60 or 120 min
- Zn^{2+} (100 µM, 24h)
- Thymol (20 µg/ml, 24h)

Cells ($5*10^6$ cells) were then snap-frozen in dry-ice ethanol bath. Cell pellets were thawed on ice and washed twice with PBS, then solubilized in lysis buffer (Pierce) containing protease inhibitor cocktail (Sigma-Aldrich, Taufkirchen, Germany. Samples were stored at -80^0C until use for western blotting.

Cell lysates were separated by 10-12% SDS-PAGE and blotted on nitrocellulose membranes. The blots were blocked with 5% nonfat-milk in triethanolamine-buffered saline (TBS) and 0.1% Tween-20 for 2hours. Then the blots were probed overnight with anti-phospho (p)-ERK, anti-ERK, anti-p-SAPK/JNK, anti-SAPK/JNK, α/ß- Tubulin (Cell Signaling, Danvers), anti p-p38, anti-p38, anti-Bcl-2, anti-Bcl-xL (Santa Cruz, Heidelberg, Germany) antibodies diluted in 5% milk in TBS and 0.1% Tween-20, washed 5 times, probed with secondary antibodies conjugated with horseradish peroxidase for 1 h at room temperature, washed final 5 times. Antibody binding was detected with the enhanced chemiluminescence (ECL) kit (Amersham, Freiburg, Germany). Densitometer scans of the blots were performed using Quantity One (BioRad, Munich, Germany).

3.9. Isolation of T and B lymphocytes from the spleen

Spleens from 8- to 12-week-old naive or GA- -treated (10%, 4 week long) C57BL/6 mice were removed aseptically, cut into pieces, a single-cell suspension of splenocytes was obtained through a cell strainer. All spleen cell preparations were resuspended in T cell isolation buffer (supplemented with 0.5% bovine serum albumin and 2 mM EDTA) and kept on ice. After blocking with anti-CD16/CD32, cells (4×10^5) were incubated in 100 µl FACS buffer (PBS plus 0.1% FCS) containing fluorochrome-conjugated antibodies at a concentration of 10 µg/ml. A total of 2×10^4 cells were analyzed. The following antibodies (BD Pharmingen, Heidelberg, Germany) were used for staining: FITC-conjugated anti-mouse

CD45, clone 30-F11 (Rat IgG_{2b}, κ), PE-conjugated anti-mouse CD19, clone 1D3 (Rat IgG_{2a}, κ) for 45 min, then cells were washed twice and resuspended in FACS buffer for flow cytometry analysis of mouse B cells.

T lymphocytes from single-cell suspensions of spleen were isolated by the negative selection column method using the Mouse Pan T isolation kits (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. Cells then were stained with FITC-conjugated anti-mouse CD3e, clone 154-2C11 (Armenian Hamster IgG_1, κ), PE-conjugated anti-mouse CD8, clone 53-6.7 (Rat IgG_{2a}, κ) (BD pharmingen) for 60 min and the cells were washed twice and resuspended in FACS buffer for flow cytometry analysis.

3.10. Phosphatidyl residue translocation

Apoptotic cell membrane scrambling was evidenced from annexin V binding to phosphatidylserine (PS) at the cell surface. To this end, the percentage of PS- translocating cells was evaluated by staining with fluorescein isothiocyanate (FITC)-conjugated Annexin V. In brief 4 $x10^5$ cells were harvested and washed twice with Annexin washing buffer (AWB, 10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, 5 mM $CaCl_2$). Cell pellet was resuspended in 100 µl of Annexin-V-Fluos labelling solution (Roche) (20µl Annexin-V-Fluos labelling reagent in 1 ml AWB), incubated for 15 min at room temperature. After washing with AWB, they were analyzed by flow cytometry.

3.11. Ceramide formation

For detection of ceramide formation mouse DCs were stained for 60 minutes at 37°C with anti-ceramide antibodies (Mouse IgM, Alexis) at a dilution of 1:10 in phosphate buffered saline (PBS) containing 0.1% fetal calf serum (FCS) {Lang, 2004 2508 /id}. After three washes with PBS/0.1% FCS, cells were stained with fluorscein isothiocyanate (FITC) - labelled goat anti-mouse IgG antibody at a dilution of 1:400 (Invitrogen, UK) in PBS/0.1% FCS for 30 min at 37°C. Unbound secondary antibodies were removed by washing the cells with PBS/0.1% FCS. Cells were then analyzed by flow cytometry (FACS Calibur, BD Biosciences).

3.12. DNA fragmentation

$5x10^5$ cells were fixed with 2% formaldehyde for 30 min on ice and then incubated with 70% ethanol for 15 min at 37°C. Cells were then treated with RNase A (40µg/ml) for 30 min at

37°C, washed and resuspended in 200µl PI (50 µg/ml, Sigma). DNA content of the samples was analyzed by flow cytometry (FACS Calibur, BD Biosciences).

3.13. Measurements of mitochodrial membrane potential

DCs ($5*10^5$cells/3ml) were either untreated or treated with xanthohumol (20µM, 24h). Cells were centrifuged at 1500 rpm, 5 min, then suspended in prewarmed PBS. The mitochondrial transmembrane potential was determined using tetramethyl-rhodamine ethyl ester (TMRE, 40 nM, Molecular Probes, Leiden, Netherlands) and then incubated for 30 min at 37^0C. Then the cells were washed twice with cold PBS before FACS analysis.

3.14. Caspase 8 and Caspase 3 activation assay

Caspase 8 and Caspase 3 activation was determined using kits (Biovision, California, USA) according to manufacturer's instruction. Briefly $1x10^6$ cells were washed twice with cold PBS, fixed and permeabilized with 'Cytofix/Cytoperm' solution and then by washing twice with 'Perm/ Wash' buffer. Then cells were stained with FITC conjugated anti-active Caspase 8 or Caspase 3 antibody in 'Perm/ Wash' buffer for 60 min. After 2 washing steps, the cells were analyzed by flow cytometry (FACS Calibur, BD Biosciences).

3.15. Immunocytochemistry

DCs were either left untreated or treated for 24 h with either Zn^{2+} (100 µM) or thymol (20 µg/ml) or xanthohumol (20 µM). DCs were then smeared onto glass slides, rinsed in PBS and fixed with 2% formaldehyde in PBS for 15 min at room temperature. After 3 washing steps with PBS for 5 min, slides were permeabilized and blocked in PBS containing 5% bovine serum albumin (BSA) and 0.3% Triton X-100 for 60 min and then incubated overnight at 4^0C with mouse monoclonal Abs to ceramide (Alexis Biochemicals) at 1:50 dilution in antibody dilution buffer (including PBS, 1%BSA and 0.3% Triton X-100). The slides were washed again three times for 5 min and then incubated with goat anti-mouse IgG-FITC (Alexis Biochemicals) in antibody dilution buffer for 90 min at 1:200 dilution at room temperature in dark. After three washing steps with high salt PBS (PBS with added NaCl to final concentration of 0.4M), nuclei were stained with DRAQ5 (10 µM, BioStatus Limitted, Shepshed Leicestershire, United Kingdom) in PBS containing 0.5%Triton X-100 for 60 min and washed final two times with PBS. Stained slides were mounted using Prolong® Gold antifade reagent (Invitrogen, Karlsruhe, Germany). Images were taken on a Zeiss LSM 5

EXCITER Confocal Laser Scanning Microscope (Carl Zeiss MicroImaging GmbH, Germany) with a ×63 Plan Apochromat objective.

3.16. Statistics

Data are provided as means ± SEM, *n* represents the number of independent experiments. All data were tested for significance using Student's unpaired two-tailed *t*-test or ANOVA and only results with $p < 0.05$ were considered statistically significant.

4. RESULTS

4.1. Thymoquinone

4.1.1. Thymoquinone inhibits maturation of LPS- stimulated DCs.

Mouse bone marrow derived DCs were treated with LPS (200 ng/ml, 24h) in the absence and presence of thymoquinone (1 – 20 μM) and then surface expression of CD11c, CD86, MHC class II, CD54 and CD40 was determined by FACS analysis. LPS increased the percentage of $CD11c^+CD86^+$, $CD11c^+$MHC class II^+, $CD11c^+CD40^+$ expressing cells (Figure 1) and CD54 expressing cells (Figure 2), effects all blunted in the presence of thymoquinone, suggesting that thymoquinone inhibits LPS- induced differentiation and maturation of DCs.

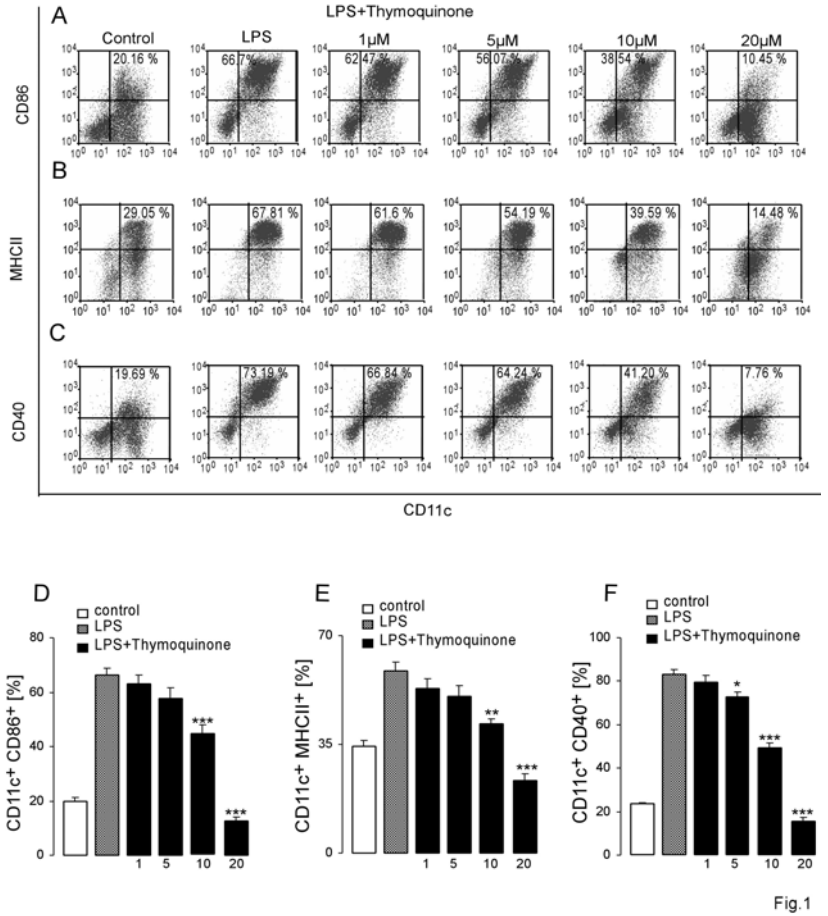

Fig.1

Figure 1: Effect of thymoquinone on LPS-induced CD86, MHC class II and CD40 surface expression in mouse DCs

A.-C. Original dot plots of $CD11c^+CD86^+$ (**A**), $CD11c^+$MHC II^+ (**B**), $CD11c^+CD40^+$ (**C**) in DCs cultured for 24 h without (control, 1st panel) or with LPS (200 ng/ml) in the absence (2d panel) and presence (3d - 6th panels) of thymoquinone (1, 5, 10, 20 μM). Numbers depict the percent of cells in the respective quadrants, acquired within the dead cell gate.
D.-F. Arithmetic means ± SEM (n = 4-6 each) of CD86, MHC class II, and CD40 expression in DCs cultured without (control, white bar) and with LPS (200 ng/ml) in the absence of (dotted bar) or presence of (black bars) thymoquinone (1 -20 μM). * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) show differences between thymoquinone-untreated and -treated LPS-stimulated DCs, ANOVA.

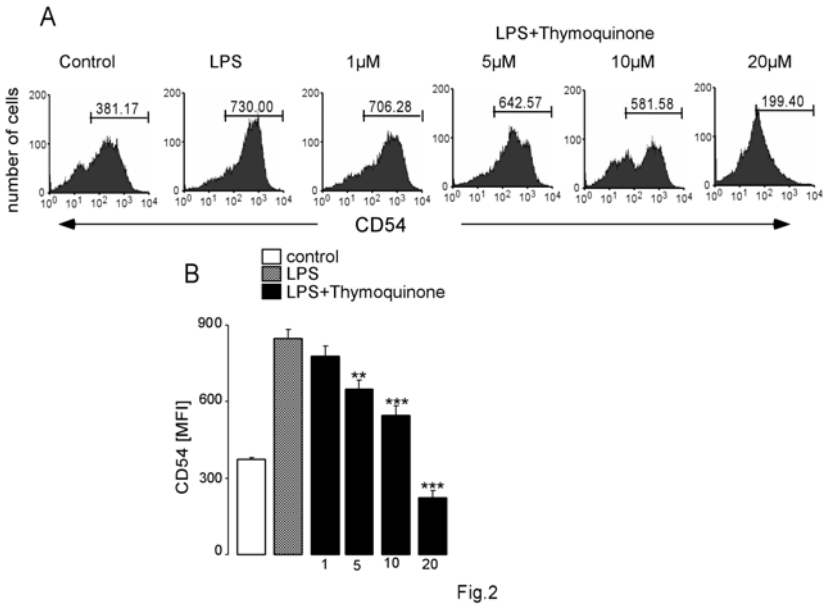

Figure 2: Effect of thymoquinone on LPS-induced CD54 surface expression in mouse DCs

A. Representative FACS histograms depicting the expression of CD54 in DCs cultured for 24 h without (control, 1st panel) or with LPS (200 ng/ml) in the absence (2d panel) and presence (3d - 6th panels) of thymoquinone (1, 5, 10, 20 µM).

B. Arithmetic means ± SEM (n = 4-6 each) of CD54 expression in DCs cultured without (control, white bar) and with LPS (200 ng/ml) in the absence of (dotted bar) or presence of (black bars) thymoquinone (1 -20 µM). ** ($p<0.01$) and *** ($p<0.001$) show differences between thymoquinone-untreated and -treated LPS-stimulated DCs, ANOVA.

4.1.2. Thymoquinone impairs cytokine secretion by LPS- stimulated DCs.

We next examined IL-10, IL-12p70 as well as TNFα formation by ELISA. DCs were stimulated with LPS (200 ng/ml, 24 h) in the absence and presence of thymoquinone (1 – 20 µM). As illustrated in Figure. 3, LPS stimulated the release of IL-10, IL-12p70 and TNFα. The effect of LPS on cytokine release was again significantly blunted in the presence of thymoquinone. The effect reached statistical significance at ≥ 1 µM thymoquinone.

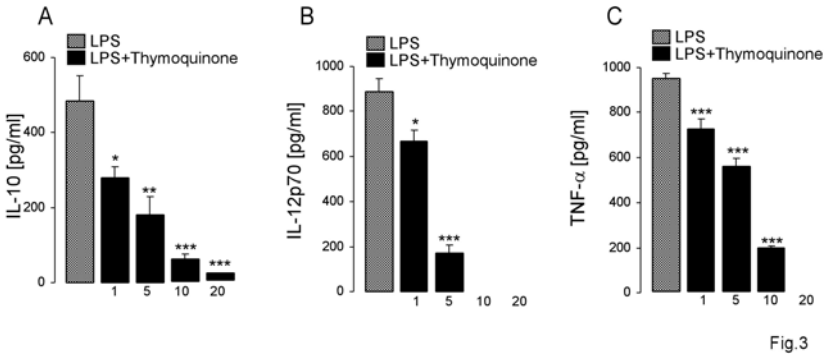

Figure 3: Effect of thymoquinone on LPS-induced formation of IL-10, IL-12 and TNF-α in mouse DCs

Concentrations of IL-10 (**A**), IL-12p70 (**B**) and TNF-α (**C**) in the supernatant of DCs cultured for 24 h without (control, white bars) and with LPS (200 ng/ml) in the absence of (dotted bar) or presence of (black bars) thymoquinone (1 -20 μM). The cytokine concentrations were determined by ELISA. Arithmetic means ± SEM were calculated from 4-9 independent experiments, each reflecting the mean of a duplicate measurement. * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) show differences between thymoquinone-untreated and -treated LPS-stimulated DCs, ANOVA.

4.1.3. Thymoquinone enhances percentage of annexin V-binding DCs.

DC apoptosis is characterized by phosphatidylserine exposure at the cell surface, which is identified by annexin V-binding in FACS analysis. To explore whether thymoquinone stimulates DC apoptosis, annexin V-binding was determined in DCs stimulated with LPS (200 ng/ml, 24 h) in the absence and presence of thymoquinone (1 – 20 μM). As illustrated in Figure. 4, LPS decreased the percentage of phospatidylserine exposing cells, an effect reversed by thymoquinine. The effect reached statistical significance at ≥ 5 μM thymoquinone.

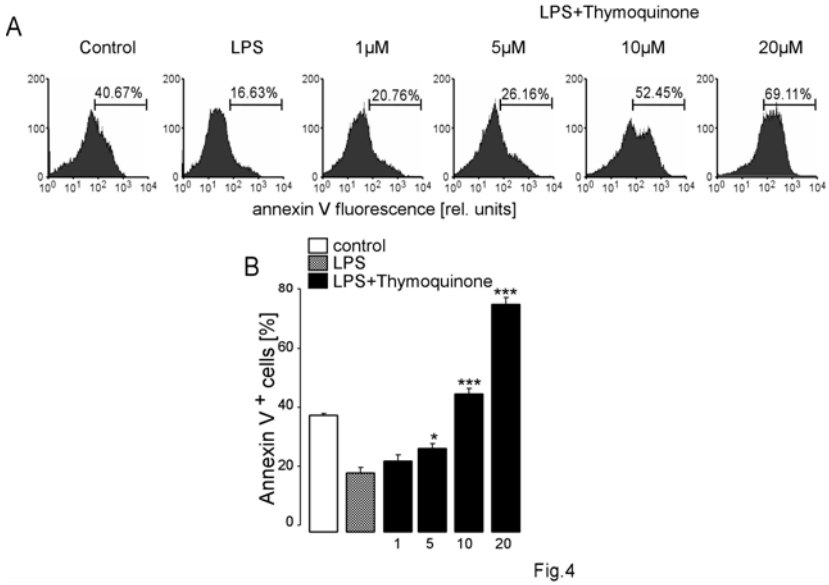

Figure 4: Effect of thymoquinone and LPS on phosphatidylserine exposure at the surface of mouse DCs

A. Representative FACS histograms depicting the expression of annexin V in DCs cultured for 24 h without (control, 1st panel) or with LPS (200 ng/ml) in the absence (2d panel) and presence (3d - 6th panels) of thymoquinone (1, 5, 10, 20 µM).

B: Arithmetic means ± SEM (n = 4-6 each) of the percentage of annexin V-positive DCs following a 24 h culture without (control, white bar) and with LPS (200 ng/ml) in the absence of (dotted bar) or presence of (black bars) thymoquinone (1 -20 µM). ** ($p<0.01$) and *** ($p<0.001$) show differences between thymoquinone-untreated and -treated LPS-stimulated DCs, ANOVA.

4.2. Gum Arabic

4.2.1. GA enhances bone marrow-derived DC differentiation and maturation

At day 8 the cultures of mouse DCs were supplemented with either LPS (200 ng/ml) or GA (0.5%) and stained for 24 h afterwards for MHC class II, CD86 and CD40 (Figure 5 and CD54 (ICAM-1) (Figure 6). Both LPS and GA increased the percentage of $CD11c^+$MHC class II^+ (Figure 5A, D), $CD11c^+CD86^+$ (Figure 5B, E), $CD11c^+CD40^+$ (Figure 5C, F) and

CD54 expressing (Figure 6) cells, indicating that, similarly to LPS, GA stimulates maturation and differentiation of DCs.

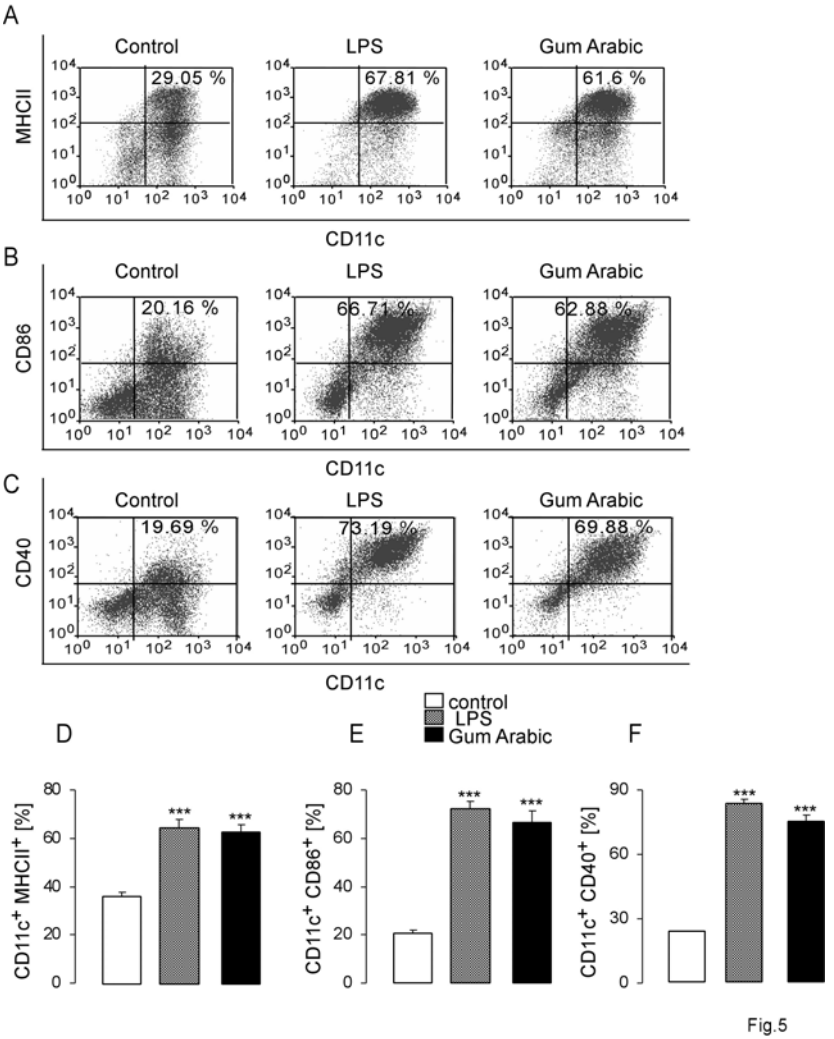

Figure 5: Effect of LPS and GA treatment on MHC class II, CD86 and CD40 surface expression in DCs

A.-C. Original dot plots of CD11c$^+$MHC class II$^+$ (**A**), CD11c$^+$CD86$^+$ (**B**), CD11c$^+$CD40$^+$ (**C**) in DCs cultured for 24 h without (control, 1st panel) or with LPS (200 ng/ml, 2^d panel) or Gum Arabic (GA, 0.5%, 3^d panel). Numbers depict the percent of cells in the respective quadrants, acquired within the dead cell gate.

D.-F. Arithmetic means ± SEM (n = 6-7) of of MHC class II (**D**), CD86 (**E**) and CD40 (**F**) expression in DCs cultured without (control, white bar) or with LPS (200 ng/ml, dotted bar) or GA (0.5%, black bars) for 24 h. *** ($p<0.001$) show differences from untreated (control) DCs, ANOVA.

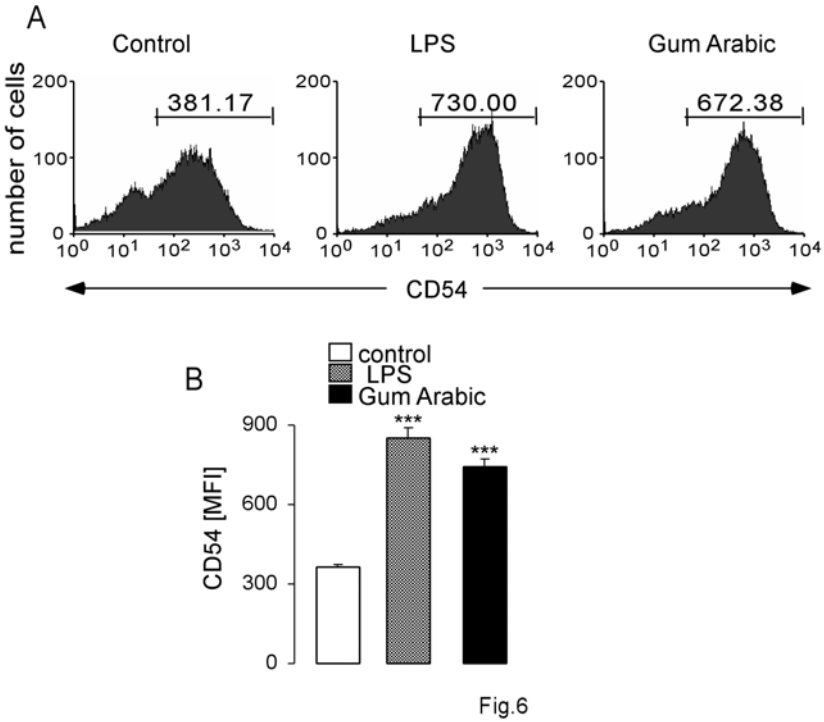

Figure 6: Effect of LPS and GA treatment on CD54 surface expression in DCs

A. Representative FACS histograms depicting the expression of CD54 in DCs cultured for 24 h without (control, 1st panel) or with LPS (200 ng/ml, 2^d panel) or Gum Arabic (GA, 0.5%, 3^d panel).

B: Arithmetic means ± SEM (n = 6 each) of CD54 expression in DCs cultured without (control, white bar) or with LPS (200 ng/ml, dotted bar) or GA (0.5%, black bars) for 24 h. *** ($p<0.001$) show differences from untreated (control) DCs, ANOVA.

4.2.2. GA enhances cytokine secretion in DCs

Cytokine production was analyzed in LPS (200 ng/ml, 24 h)- or GA (0.5%, 24 h)-stimulated DCs. As illustrated in Figure 7, both LPS and GA stimulated the secretion of IL-10, IL-12p70, IL-6 and TNFα. GA-stimulated DCs secreted more IL-10 than LPS-stimulated cells (Figure 7A) and in contrast, the effect of LPS on IL-12p70 (Figure 7B) and IL-6 (Figure 7C) production was significantly more pronounced than the effect of GA.

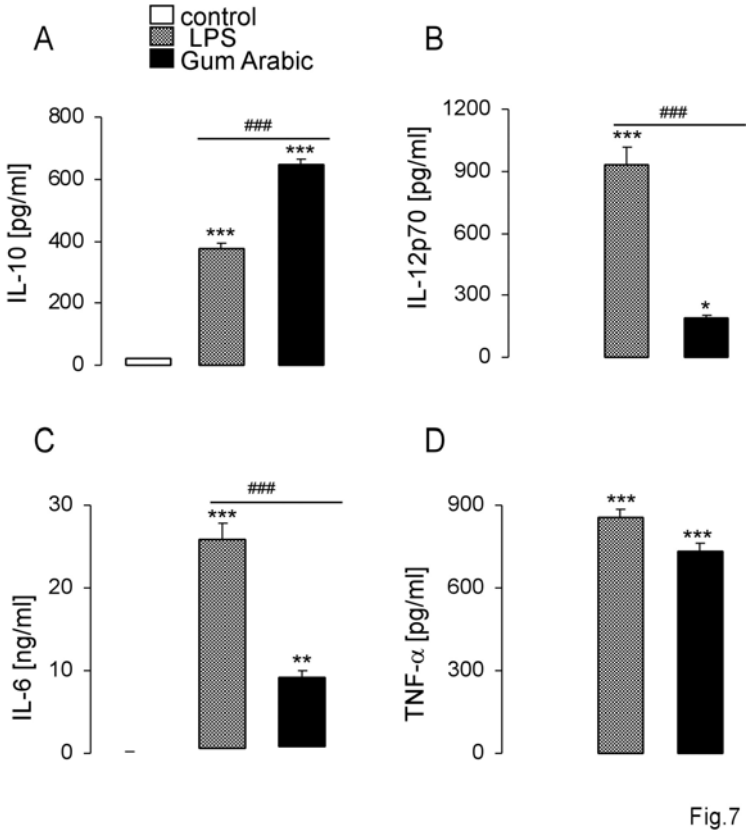

Figure 7: Effect of LPS and GA treatment on the formation of IL-10, IL-12p70, IL-6 and TNF-α

Concentrations of IL-10 (**A**), IL-12p70 (**B**), IL-6 (**C**) and TNF-α (**D**) in the supernatants of DCs cultured without (control, white bars) or with LPS (200 ng/ml, dotted bar) or GA (0.5%, black bars) for 24 h. The cytokine concentrations were determined by ELISA. Arithmetic means ± SEM were calculated from 6-8 independent experiments, each reflecting the mean of a duplicate measurement. * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) show differences from untreated (control) group, ### ($p<0.001$) denotes differences between GA and LPS, ANOVA.

4.2.3. GA decreases phagocytic capacity in DCs

DC maturation is characterized by the loss of phagocytic capacity and LPS-induced maturation of DCs is known to down-regulate the FITC- dextran uptake {Nakahara, 2004 166 /id}. Accordingly, the phagocytic capacity of unstimulated, LPS (200 ng/ml, 24 h)- or GA (0.5%, 24 h)- stimulated DCs was analyzed. As shown in Figure 8, FITC-dextran uptake was similarly impaired by GA and LPS, confirming that GA indeed promoted maturation of DCs.

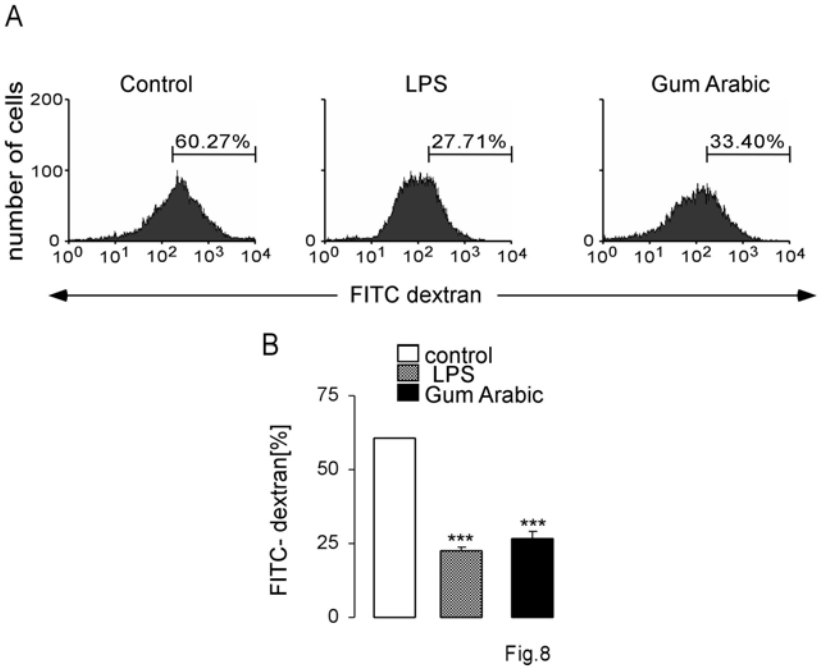

Figure 8: LPS and GA decrease phagocytic capacity of DCs

A. Representative FACS histograms depicting the percentage of the uptake of FITC-dextran after 3h in DCs cultured for 24 h without (control, 1st panel) or with LPS (200 ng/ml, 2d panel) or Gum Arabic (GA, 0.5%, 3d panel).

B. Bar diagram representing mean percent (± SEM; n=4) of FITC-dextran uptake by DCs cultured without (control, white bars) and with LPS (200 ng/ml, dotted bar) or GA (0.5%, black bars) for 24 h. *** ($p<0.001$) show differences from untreated (control) group, ANOVA.

4.2.4. Stimulation with GA leads to upregulation of extracellular signal-regulated kinase (ERK1 and ERK2) in DCs

LPS stimulation has been shown to activate mitogen-induced protein kinases (MAPKs) in DCs {Nakahara, 2004 166 /id;An, 2002 1452 /id}. To get insight into GA-induced signaling, we examined phosphorylation (and thus activation) status of ERK1/2, p38 and SAPK/JNK MAPKs in DCs stimulated with either LPS or GA (Figure. 9). LPS stimulation fostered p38, ERK1/2 and JNK activation, while GA strongly affected both ERK1 and ERK2 phosphorylation.

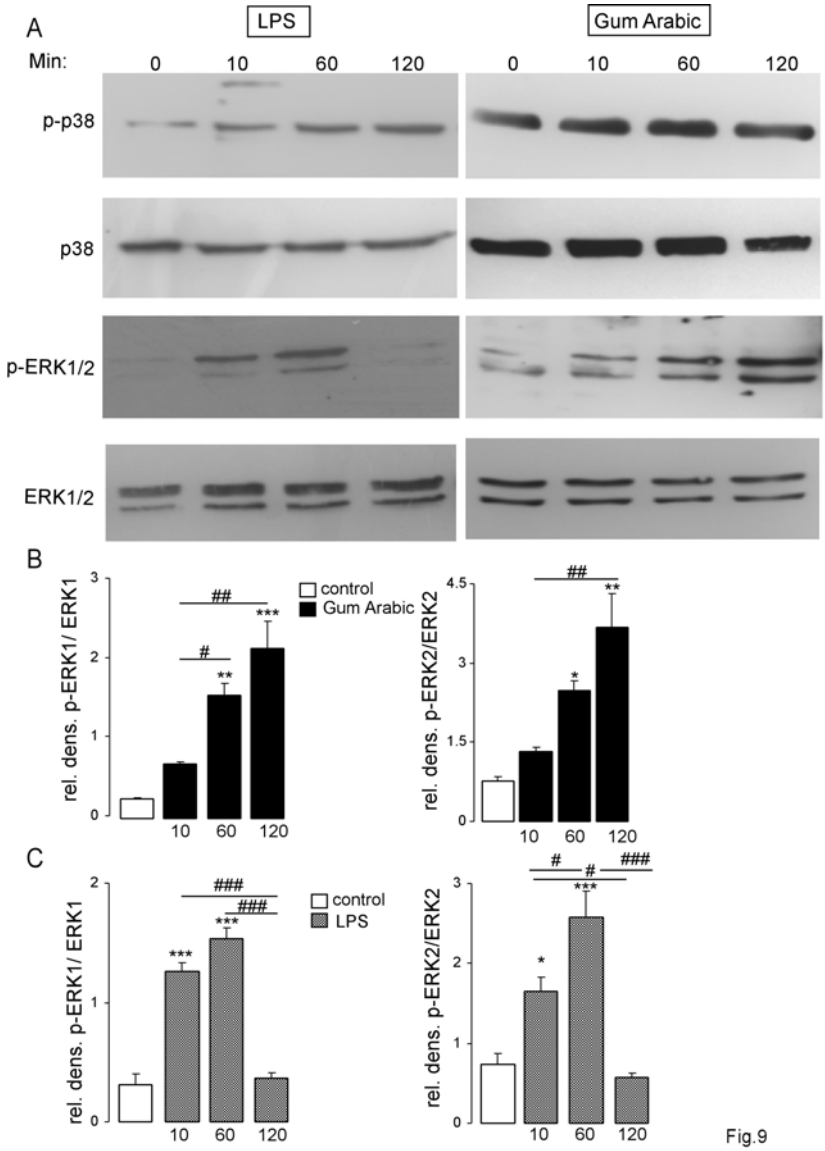

Figure 9: GA enhances the phosphorylation of ERK1 and ERK2 in DCs

A. DCs were stimulated with LPS (200 ng/ml) or GA (0.5%) for the indicated time: 10, 60, 120 min or left untreated (control). Protein extracts were analyzed by direct Western blotting

using antibodies directed to phosphorylated (p) p38, p-ERK, p-SAPK/JNK. Equal protein loading was controlled by anti-p38, ERK, and SAPK/JNK antibodies. One representative experiment out of three is shown.

B., C. Arithmetic means ± SEM (n = 3) of the abundance of phosphorylated ERK1 (left) and ERK2 (right) as the ratio to total ERK1 (ERK2) at 0 (control), 10, 60 and 120 min of incubation with either GA (0.5%, B) or LPS (C). * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) indicate significant difference from control, # ($p<0.05$) and ## ($p<0.01$) indicate significant difference from 10 min incubation point; ANOVA.

4.2.5. GA treatment leads to a higher percentage of splenic $CD8^+$ T cells and B cells

Finally, T and B cells were isolated from spleen of naive or GA (10%, 4 weeks)-treated mice. The percentage of $CD3^+CD8^+$ T cells and $CD45^+CD19^+$ B cells was enhanced in mice treated with GA (Figure 10).

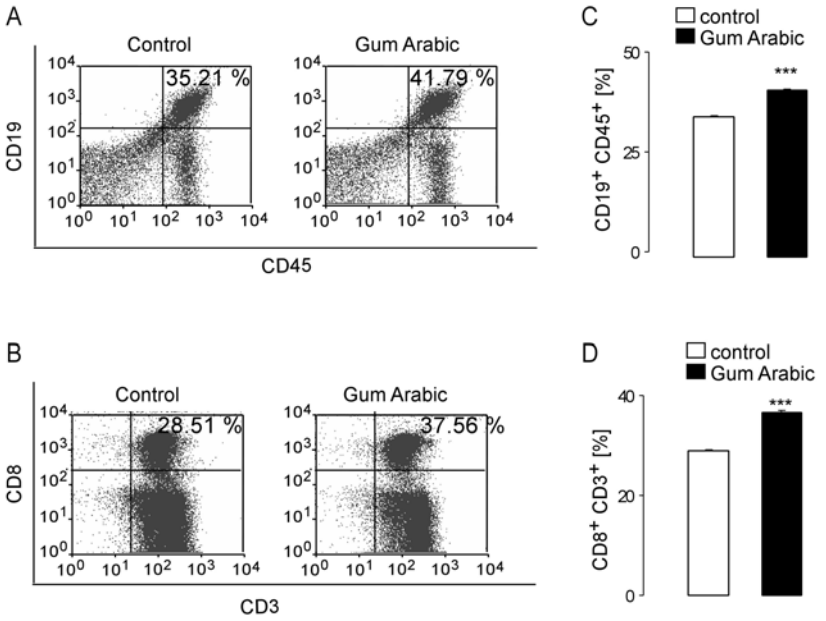

Figure 10: GA increases the percentage of splenic $CD8^+$ T cells and B cells

A., B. Original dot plots of $CD45^+CD19^+$ B cells (**A**) and $CD3^+CD8^+$ T cells (**B**) in the spleens of mice untreated (control, 1st panel) or treated for 4 weeks with GA (10%, 2d panel).

Numbers depict the percent of cells in the respective quadrants, acquired within the mononuclear cell gate.

C., D. Arithmetic means ± SEM (n = 4-9) of the percentage of $CD19^+CD45^+$ (**C**) and $CD3^+CD8^+$ T cells (**D**) in the spleen of mice untreated (control, white bar) or treated for 4 weeks with GA (10%, black bar) *** ($p<0.001$) show differences from untreated (control) group (two-tailed unpaired *t*-test).

4.3. Zn^{2+}, xanthohumol and thymol

4.3.1. Effect of Zn^{2+}, xanthohumol and thymol on ceramide formation

DCs were cultured from either wild type mice or mice lacking functional acid sphingomyelinase ($ASM^{-/-}$). The cells were grown in GM-CSF containing media for 8 days and in the following exposed for 24 hours to either Zn^{2+} (100 µM) or xanthohumol (20 µM) or thymol (20 µg/ml). As illustrated in Figure 11, administration of these nutrients within 24 h stimulated ceramide formation in DCs from wild type mice but not in DCs from $ASM^{-/-}$ mice. The observation revealed a stimulating effect of the nutrients on the acid sphingomyelinase of wild type mice. Figure 12 shows the dose dependence of the effect of these nutrients on ceramide production. LPS, which leads to DC activation through TLR4, is known to result in enhanced DC survival by inhibition of DC apoptosis {Banchereau, 2000 50 /id}. Thus, the production of ceramide was slightly, but significantly, decreased by LPS treatment (100 nM, 24 h). Immunocytochemical analysis confirmed enhanced ceramide production induced by either Zn^{2+} or xanthohumol or thymol in wild type but not in $ASM^{-/-}$ DCs as indicated in Figure 13.

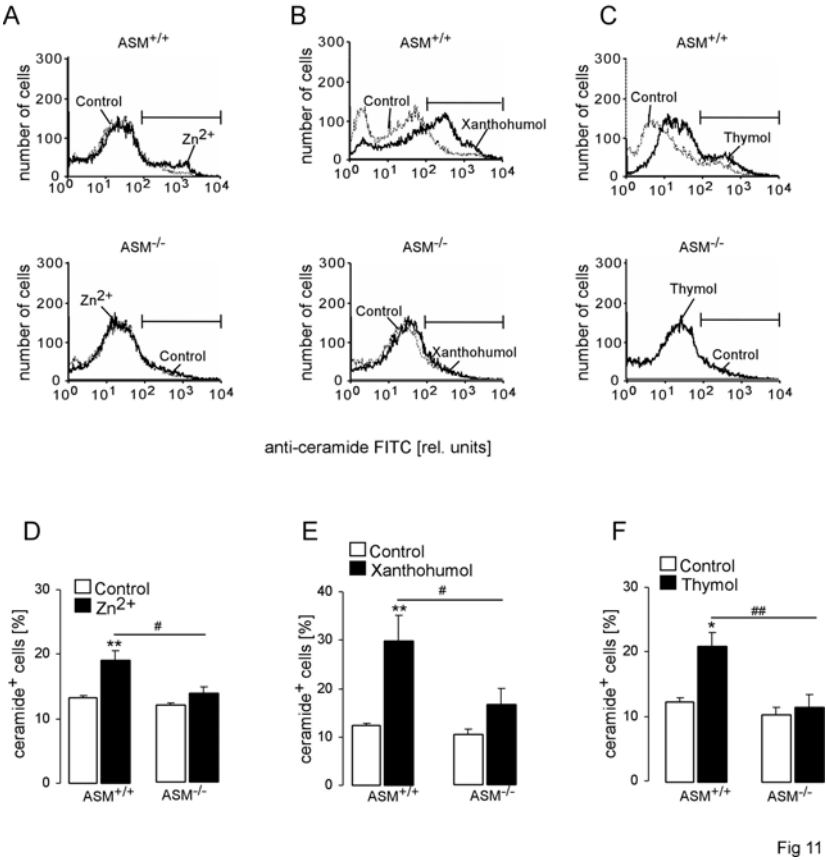

Fig 11

Figure 11: Effect of Zn^{2+}, xanthohumol and thymol on ceramide formation in DCs from wild type and $ASM^{-/-}$ mice

A.-C. Histograms of anti-ceramide FITC-coupled-antibody binding as obtained by FACS analysis in a representative experiment on wild type (upper panels) and $ASM^{-/-}$ (lower panels) DCs which were either left untreated (control, dotted line) or incubated for 24 h with Zn^{2+} (100 µM, black line, **A**) or xanthohumol (20 µM, black line, **B**) or thymol (20 µg/ml, black line, **C**).

D.-F. Arithmetic means (n = 4-6) of the percentage of wild type (left bars) and $ASM^{-/-}$ (right bars) DCs presenting ceramide at the cell surface. Ceramide formation is shown prior to (control, white bars) and 24h following (black bars) treatment with either Zn^{2+} (100 µM, **D**)

or xanthohumol (20 µM, **E**) or thymol (20 µg/ml, **F**). * ($p<0.05$) and ** ($p<0.01$) represent significant difference from wild type cells under control conditions and # ($p<0.05$), ## ($p<0.01$) represent difference between wild type and $ASM^{-/-}$ DCs, ANOVA.

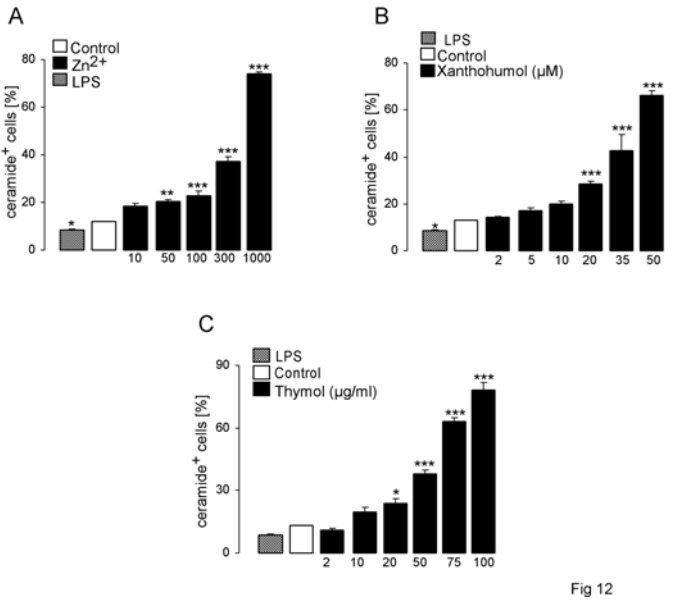

Figure 12: Dose dependence of the effect of Zn^{2+}, xanthohumol and thymol on ceramide production

Dose dependent effect of Zn^{2+} (**A**), xanthohumol (**B**) and thymol (**C**) on ceramide formation. Arithmetic means (n = 4-5) of the percentage of wild type DCs presenting ceramide at the cell surface. Ceramide formation is shown prior to (control, white bar) and 24h following (black bars) treatment with either Zn^{2+} (10-1000 µM,) or xanthohumol (2-50 µM,) or thymol (2-100 µg/ml,) or LPS (100 nM, dotted bars). * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) represent significant difference from control condition, ANOVA.

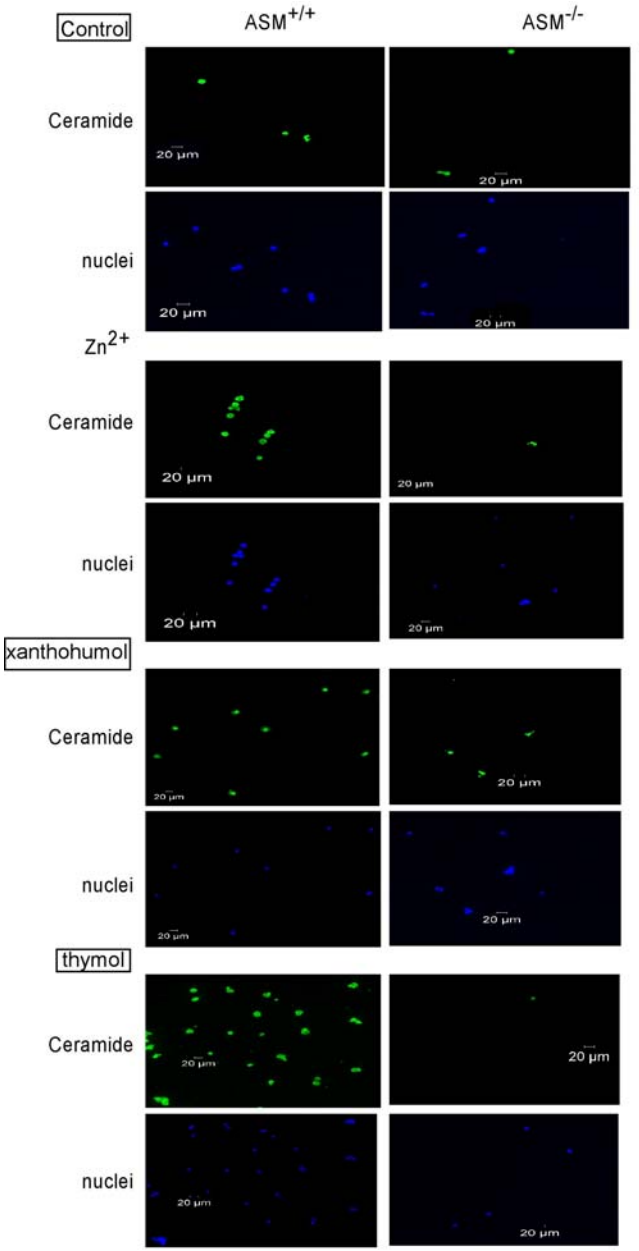
Control
ASM+/+
ASM-/-
Ceramide
nuclei
Zn2+
Ceramide
nuclei
xanthohumol
Ceramide
nuclei
thymol
Ceramide
nuclei
20 µm

Fig 13

Figure 13: Immunohistochemical analysis of nutrient -induced ceramide formation in wild type and ASM$^{-/-}$ DCs

Immunohistochemistry of anti-ceramide FITC-coupled-antibody binding and nuclei staining in a representative experiment on wild type (left panels) and ASM$^{-/-}$ DCs (right panels) either untreated (control, 1st, 2nd panels) or incubated for 24 h with Zn^{2+} (100 µM, 3rd, 4th panels) or xanthohumol (20 µM, 5th, 6th panels) or thymol (20 µg/ml, 7th, 8th panels).

4.3.2. Effect of Zn^{2+}, xanthohumol and thymol on DNA fragmentation

The experiments were performed to elucidate, whether Zn^{2+}, xanthohumol and thymol lead to DNA fragmentation, a hallmark of apoptosis. As illustrated in Figure 14, the exposure to these nutrients was indeed followed by an increase of cells in the sub-G1 phase, indicating DNA fragmentation. Figure 15 shows the dose dependence of the nutrient effect on DNA fragmentation. Again, LPS-stimulated DCs showed a decreased DNA fragmentation if compared to unstimulated cells (Figure 15). The nutrients did not elicit DNA fragmentation in DCs derived from ASM$^{-/-}$ mice (Figure 14), indicating that ASM-mediated production of ceramide may trigger DNA fragmentation in DCs.

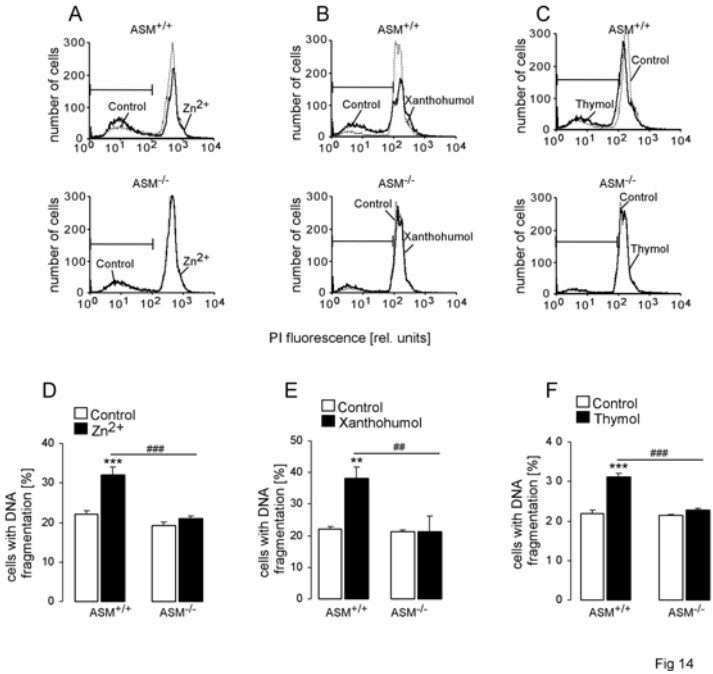

Fig 14

Figure 14: Effect of Zn^{2+}, xanthohumol and thymol on DNA fragmentation in DCs from wild type and $ASM^{-/-}$ mice

A.-C. Histograms of DNA content in sub-G1 fraction obtained by FACS analysis in a representative experiment on wild type (upper panels) and $ASM^{-/-}$ (lower panels) DCs either untreated (control, dotted line) or incubated for 24 h with Zn^{2+} (100 µM, black line, **A**) or xanthohumol (20 µM, black line, **B**) or thymol (20 µg/ml, black line, **C**).

D.-F. Arithmetic means (n = 4-6) of the percentage of wild type (left bars) and $ASM^{-/-}$ (right bars) DCs with fragmented DNA. DNA fragmentation is shown prior to (control, white bars) and 24h following (black bars) treatment with either Zn^{2+} (100 µM, **D**) or xanthohumol (20 µM, **E**) or thymol (20 µg/ml, **F**). ** ($p<0.01$) and *** ($p<0.001$) represent significant difference from wild type cells under control conditions and $^{\#\#}$ ($p<0.01$) and $^{\#\#\#}$ ($p<0.001$) represent difference between wild type and $ASM^{-/-}$ DCs, ANOVA.

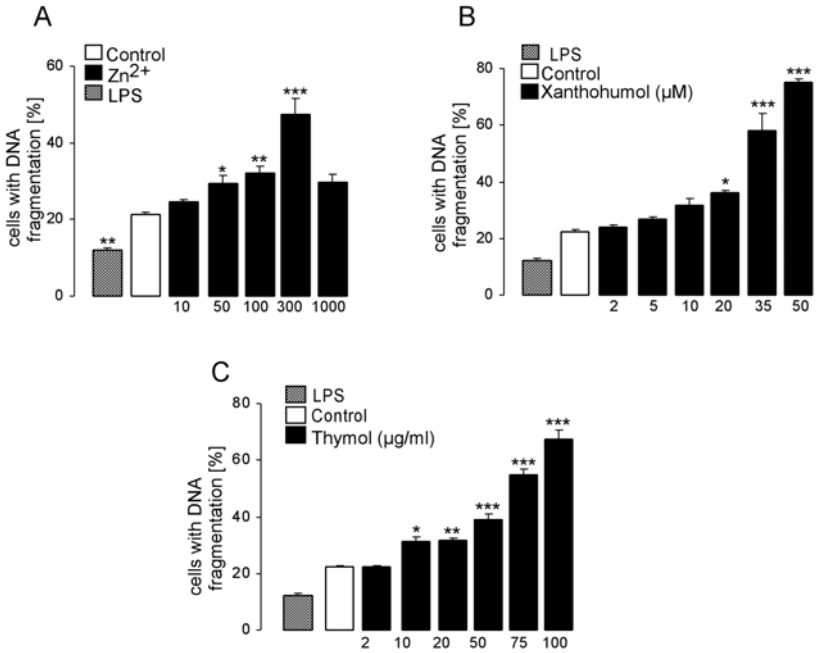

Fig 15

Figure 15: Dose dependence of the effect of Zn^{2+}, xanthohumol and thymol on DNA fragmentation

Dose dependent effect of Zn^{2+} (**A**), xanthohumol (**B**) and thymol (**C**) on DNA fragmentation. Arithmetic means (n = 4-5) of the percentage of wild type DCs with fragmented DNA. DNA fragmentation is shown prior to (control, white bar) and 24 h following (black bars) treatment with either Zn^{2+} (10-1000 µM, **A**) or xanthohumol (2-50 µM, **B**) or thymol (2-100 µg/ml, **C**) or LPS (100 nM, dotted bars). * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) represent significant difference from control condition, ANOVA.

4.3.3. Effect of xanthohumol and thymol on caspase 8 activity

As ceramide is known to stimulate suicidal cell death, a following series was performed to elucidate the effect of nutrients on caspase activation. The administration of xanthohumol and thymol, but not Zn^{2+}, was indeed followed by activation of caspase 8 in wild type DCs, an effect reaching statistical significance at the concentration of xanthohumol (20 µM, Figure 16E) and thymol (50 µg/ml, Figure 16F). In contrast, no stimulation of caspase 8 activation was observed in DCs derived from $ASM^{-/-}$ mice (Figure 16A-D), indicating that the effect of xanthohumol and thymol on caspase 8 activation was next to stimulation of ceramide formation.

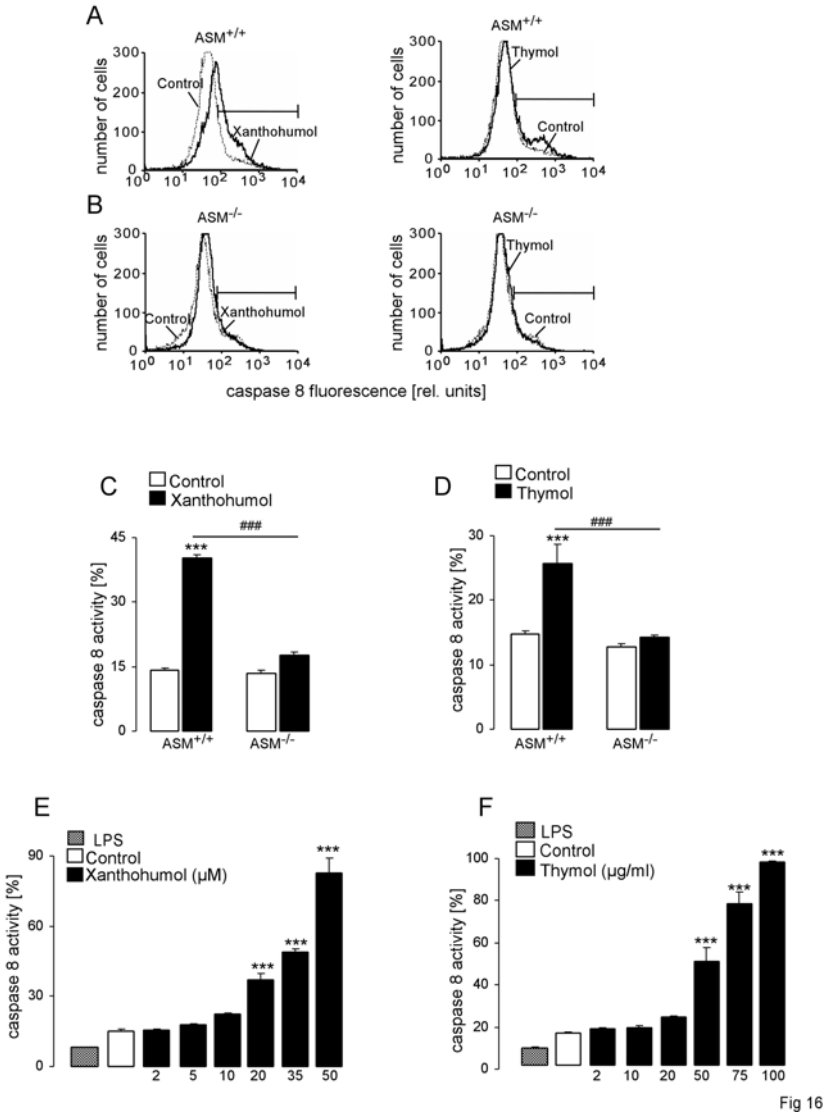

Figure 16: Effect of xanthohumol, thymol on caspase 8 activity

A., B. Histograms of caspase 8 activity as obtained by FACS analysis in a representative experiment on wild type (upper panels) and $ASM^{-/-}$ (lower panels) DCs either untreated

(control, dotted line) or incubated for 24 h with either xanthohumol (20 µM, black line, **A**) or thymol (20 µg/ml, black line, **B**).

C., D. Arithmetic means (n = 4-6) of the percentage of wild type (left bars) and $ASM^{-/-}$ (right bars) DCs with activated caspase 8. Caspase activation is shown prior to (control, white bars) and 24h following (black bars) treatment with either xanthohumol (20 µM, **C**) or thymol (20 µg/ml, **D**). *** ($p<0.001$) represents significant difference from wild type cells under control conditions and ### ($p<0.001$) represents difference between wild type and $ASM^{-/-}$ DCs, ANOVA.

E., F. Dose dependent effect of xanthohumol and thymol on caspase 8 activation. Arithmetic means (n = 4-6) of the percentage of wild type DCs with activated caspase 8. Caspase 8 activity is shown prior to (control, white bar) and 24h following (black bars) treatment with either xanthohumol (2-50 µM, **E**) or thymol (2-100 µg/ml, **F**) or LPS (100 nM, dotted bars). *** ($p<0.001$) represents significant difference from control condition, ANOVA.

4.3.4. Effect of xanthohumol and thymol on caspase 3 activity

Similar to caspase 8, caspase 3 activity was activated by administration of xanthohumol and thymol in wild type DCs. Again, xanthohumol at concentrations of 20 µM (Figure 17E) or thymol of 20 µg/ml (Figure 17F) stimulated the caspase 3 only in wild type DCs but not in DCs from $ASM^{-/-}$ mice (Figure 17A-D). Thus, activation of caspase 3, similar to that of caspase 8, was significantly stimulated by xanthohumol and thymol by a mechanism requiring functional acid sphingomyelinase.

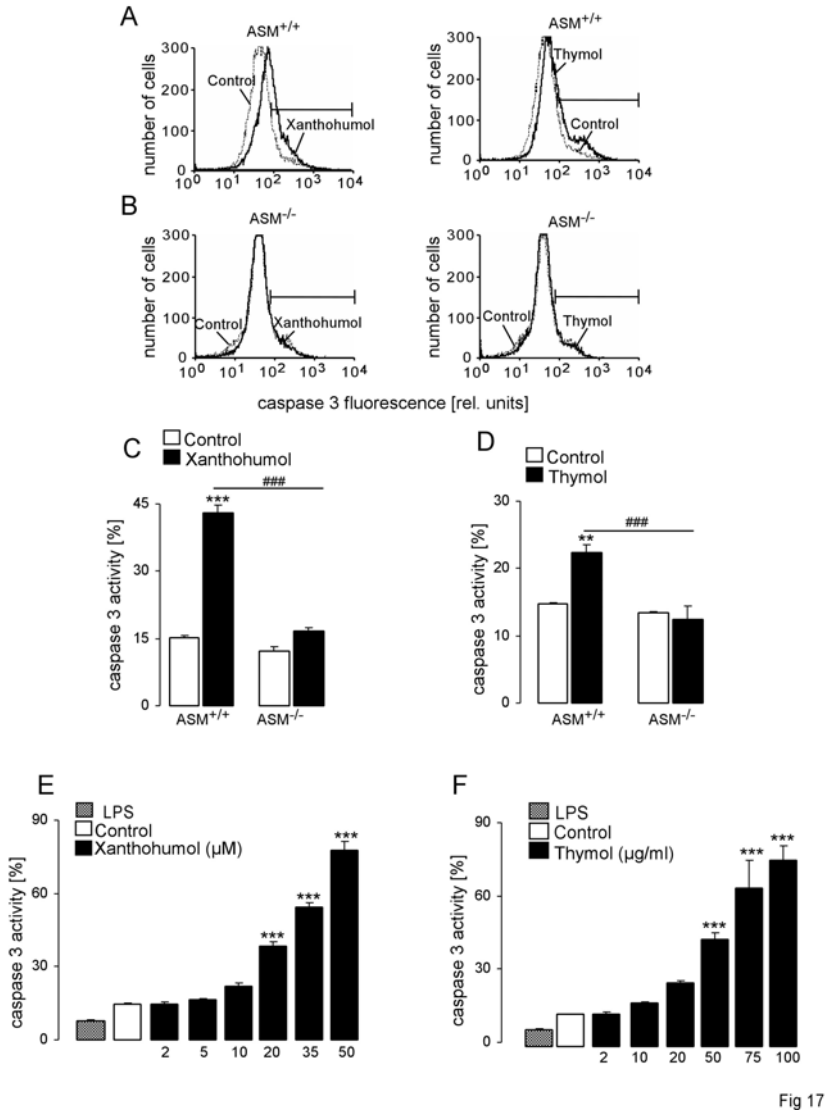

Figure 17: Effect of xanthohumol and thymol on caspase 3 activity

A., B. Histograms of caspase 3 activity as obtained by FACS analysis in a representative experiment on wild type (upper panels) and ASM$^{-/-}$ (lower panels) DCs either untreated

(control, dotted line) or incubated for 24 h with either xanthohumol (20 µM, black line, **A**) or thymol (20 µg/ml, black line, **B**).

C., D. Arithmetic means (n = 4-6) of the percentage of wild type (left bars) and $ASM^{-/-}$ (right bars) DCs with activated caspase 3. Caspase activation is shown prior to (control, white bars) and 24h following (black bars) treatment with either xanthohumol (20 µM, **C**) or thymol (20 µg/ml, **D**). ** ($p<0.01$), *** ($p<0.001$) represent significant difference from wild type cells under control conditions and $^{###}$ ($p<0.001$) represents difference between wild type and $ASM^{-/-}$ DCs, ANOVA.

E., F. Dose dependent effect of xanthohumol and thymol on caspase 3 activation. Arithmetic means (n = 4-6) of the percentage of wild type DCs with activated caspase 3. Caspase 3 activity is shown prior to (control, white bar) and 24h following (black bars) treatment with either xanthohumol (2-50 µM, **E**) or thymol (2-100 µg/ml, **F**) or LPS (100 nM, dotted bars). *** ($p<0.001$) represent significant difference from control condition, ANOVA.

4.3.5. Effect of Zn^{2+}, xanthohumol and thymol on cell membrane scrambling

A final series of experiments elucidated the effect of Zn^{2+}, xanthohumol and thymol on phosphatidylserine exposure of the cell membrane. Cell membrane scrambling with break down of phosphatidylserine asymmetry is a further hallmark of apoptosis. The phosphatidylserine exposure at the cell surface was determined utilizing binding of FITC-labelled annexin V. As shown in Figure 18 exposure of DCs to Zn^{2+}, xanthohumol and thymol was followed by an increase of the percentage of annexin V binding cells in wild type but not in $ASM^{-/-}$ DCs. Figure 19 shows the dose dependence of the effect of these nutrients on annexin V binding.

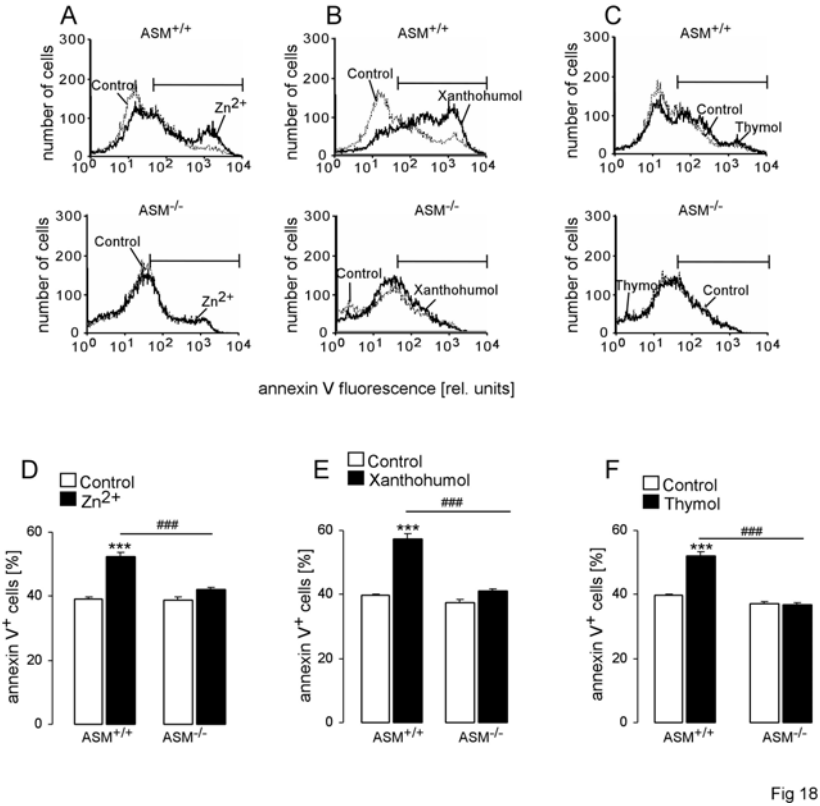

Fig 18

Figure 18: Effect of Zn^{2+}, xanthohumol and thymol on cell membrane scrambling

A.-C. Histograms of annexin V binding as obtained by FACS analysis in a representative experiment on wild type (upper panels) and $ASM^{-/-}$ (lower panels) DCs either untreated (control, dotted line) or incubated for 24 h with Zn^{2+} (100 µM, black line, **A**) or xanthohumol (20 µM, black line, **B**) or thymol (20 µg/ml, black line, **C**).

D.-F. Arithmetic means (n = 4-6) of the percentage of wild type (left bars) and $ASM^{-/-}$ (right bars) DCs with annexin V binding. Annexin V binding is shown prior to (control, white bars) and 24h following (black bars) treatment with either Zn^{2+} (100 µM, **D**) or xanthohumol (20 µM, **E**) or thymol (20 µg/ml, **F**). ***($p<0.001$) represents significant difference from wild type cells under control conditions and $^{###}$($p<0.001$) represents difference between wild type and $ASM^{-/-}$ DCs, ANOVA.

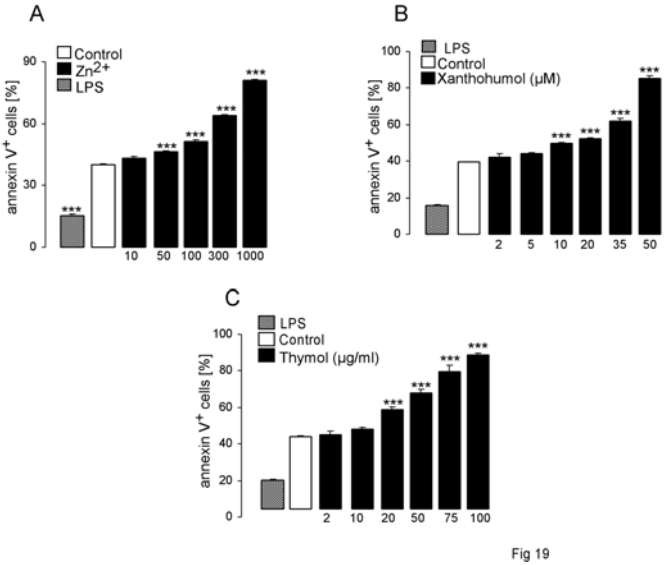

Figure 19: Dose dependence of the effect of Zn^{2+}, xanthohumol and thymol on cell membrane scrambling

Dose dependent effect of either Zn^{2+} (**A**) or xanthohumol (**B**) or thymol (**C**) on annexin V binding. Arithmetic means (n = 4-5) of the percentage of wild type DCs with annexin V binding. Annexin V binding is shown prior to (control, white bars) and 24h following (black bars) treatment with either Zn^{2+} (10-1000 µM, **A**) or xanthohumol (2-50 µM, **B**) or thymol (2-100 µg/ml, **C**) or LPS (100 nM, dotted bars). *** ($p<0.001$) represents significant difference from control condition, ANOVA.

4.3.6. Effect of C2-ceramide on DC apoptosis

The effect of Zn^{2+}, xanthohumol and thymol on DC apoptosis was mimicked by addition of 10 µM C2-ceramide, which led within 24 hours to a marked stimulation of DC apoptosis in both wild type and $ASM^{-/-}$ DCs. As illustrated in Figure 20, administration of C2-ceramide stimulated DNA fragmentation, caspase 8 and 3 activity and membrane scrambling in DCs from both wild type and from $ASM^{-/-}$ mice.

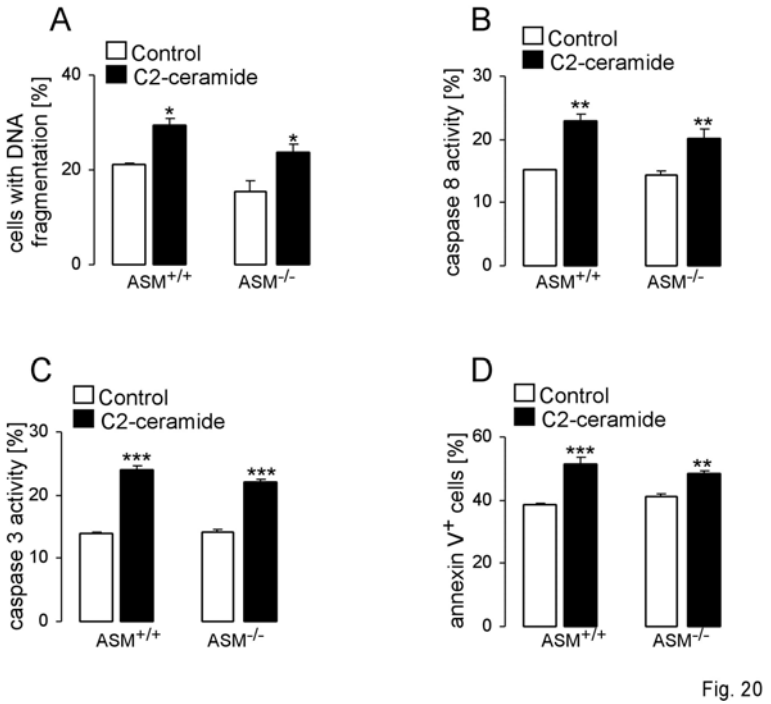

Fig. 20

Figure 20: Effect of C2-ceramide on DC apoptosis

Arithmetic means (n = 3) of the percentage of wild type (left bars) and $ASM^{-/-}$ (right bars) DCs presenting DNA fragmentation (**A**), caspase 8 activity (**B**), caspase 3 activity (**C**) and membrane scrambling (**D**). Apoptotic DCs are shown prior to (control, white bars) and 24h following (black bars) treatment with C2-ceramide (10 μM). * ($p<0.05$) and ** ($p<0.01$) represent significant difference between control and treated cells, ANOVA.

4.3.7. Effect of Zn^{2+} and thymol on Bcl-2 and Bcl-xL protein abundance

In addition, to determine whether Zn^{2+}, xanthohumol and thymol affect proteins of the Bcl-2 family, we examined the expression levels of anti-apoptotic Bcl-2 and Bcl-xL proteins by Western blot analysis. As shown in Figure 21, treatment of DCs with either Zn^{2+} or thymol (but not xanthohumol) resulted in reduction of the abundance of anti-apoptotic Bcl-2 and Bcl-xL proteins in wild type but not in $ASM^{-/-}$ DCs.

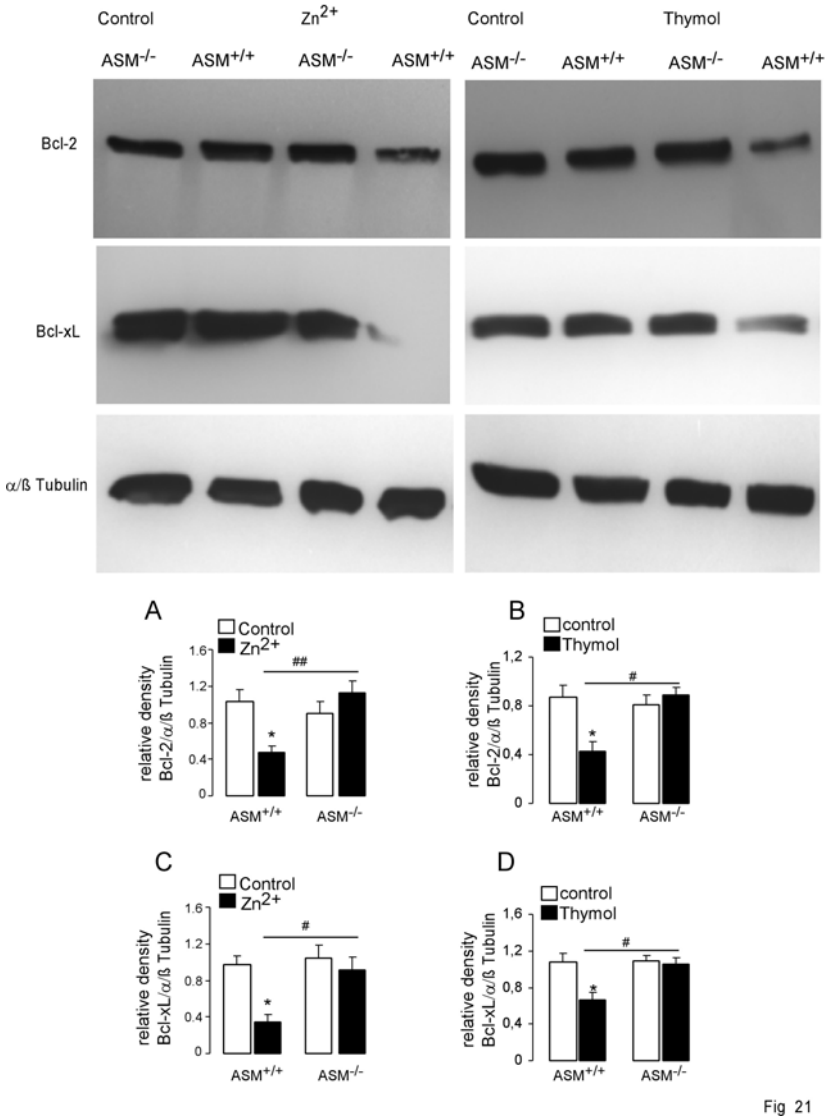

Fig 21

Figure 21: Effect of Zn^{2+} and thymol on Bcl-2 and Bcl-xL protein abundance

A.,B. Original Western blot of DCs from wild type and $ASM^{-/-}$ mice, which were treated with either Zn^{2+} (100 µM, 24 h, **A**) or thymol (20 µg/ml, 24 h, **B**) or left untreated (control). Protein extracts were analyzed by direct Western blotting using antibodies directed to Bcl-2 or to Bcl-xL. Protein loading was controlled by anti-α/β-tubulin antibody.

C.-F. Arithmetic mean ±SEM (n = 3) of ratio of Bcl-2:α/β-tubulin (C, D)or Bcl-xL:α/β-tubulin (E, F) in wild type (left bars) and $ASM^{-/-}$ (right bars) DCs. The abundance of Bcl-2 or Bcl-xL is shown prior to (control, white bars) and 24h following (black bars) treatment with either Zn^{2+} (100 µM, **C**, **E**) or thymol (20 µg/ml, **D**, **F**). * ($p<0.05$) indicates significant difference between treated and untreated wild type DCs. $^{\#}(p<0.05)$ and $^{\#\#}(p<0.01)$ indicate difference between wild type and $ASM^{-/-}$ DCs, ANOVA.

4.3.8. Effect of xanthohumol on mitochodrial menbrane potential of DCs

Mitochondrial dysfunction is characterized by a marked reduction in mitochondrial membrane potential (Δψm). Thus, we determined whether DCs treated with Zn^{2+}, xanthohumol or thymol loose their mitochondrial potential. Flow cytometric analysis as indicated in Figure 22, administration of xanthohumol (but not Zn^{2+} or thymol) led within 24 h to mitochondrial membrane depolarization in wild type DCs. However, xanthohumol led to the loss of mitochondrial potential also in $ASM^{-/-}$ DCs and thus, this effect of xanthohumol is not mediated by ASM-induced ceramide production.

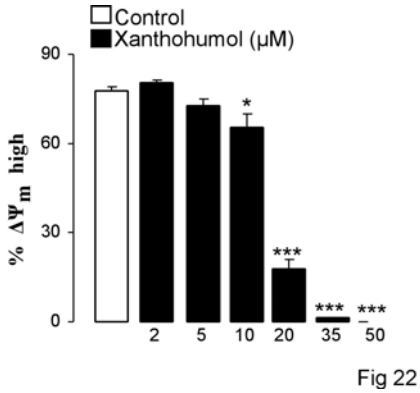

Figure 22: Effect of xanthohumol on mitochodrial menbrane potential of wild type DCs

Arithmetic means (n = 4 each) of percent of wild type DCs with high mitochondrial membrane potential under control conditions (control, white bar) or after 24 h incubation with

xanthohumol (2-50 µM, black bars). * ($p<0.05$), *** ($p<0.001$) indicate significant difference from control group, ANOVA.

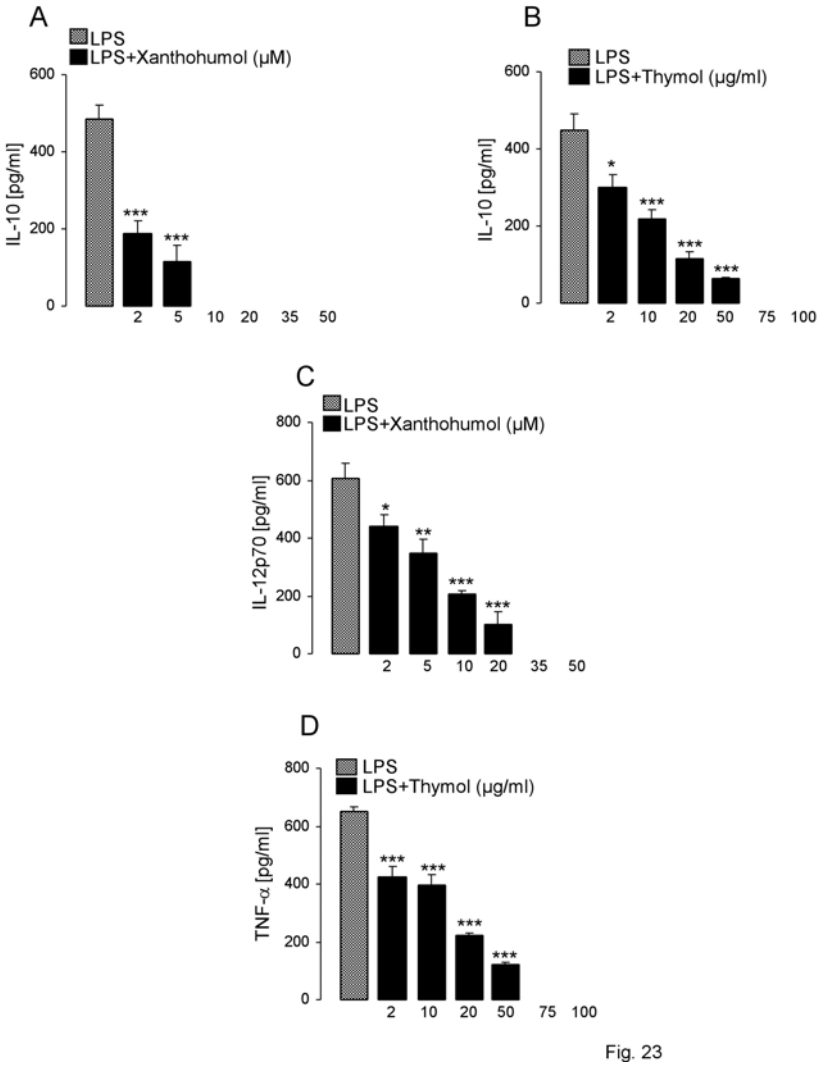

Fig. 23

Figure 23: Effect of xanthohumol and thymol on cytokine secretion in LPS- treated DCs

A., B. Arithmetic means (n = 4 each) of IL-10 secretion by LPS-stimulated (100 ng/ml, 24h) DCs untreated (grey bars) or treated (black bars) with either xanthohumol (2-50 µM, **A**) or thymol (2-100 µg/ml, **B**). * ($p<0.05$) and *** ($p<0.001$) indicate significant difference from LPS-stimulated DCs, ANOVA..

C. Arithmetic means (n = 4 each) of the IL-12p70 formation in LPS-stimulated (100 ng/ml, 24h) DCs untreated (grey bar) or treated with xanthohumol (2-50 µM, black bars). * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) indicate significant difference from LPS-stimulated DCs, ANOVA.

D. Arithmetic means (n = 4 each) of the TNFα formation in LPS-stimulated (100 ng/ml, 24h) DCs untreated (grey bar) or treated with thymol (2-100 µg/ml). *** ($p<0.001$) indicates significant difference from LPS-stimulated DCs, ANOVA.

4.3.9. Xanthohumol and thymol impaired and Zn^{2+} enhanced cytokine secretion by LPS- stimulated DCs.

We next examined whether Zn^{2+}, xanthohumol and thymol influence the production of cytokines from DCs. IL-10, IL-12p70 as well as TNFα formation was analyzed by ELISA. DCs were stimulated with LPS (200 ng/ml, 24 h) in the absence and presence of either Zn^{2+} (50-1000 µM), xanthohumol (20 µM) or thymol (20 µg/ml). As illustrated in Figure 23, LPS-induced release of IL-10 was significantly blunted by xanthohumol and thymol. Moreover, xanthohumol decreased the production of IL-12p70 and thymol decreased the production of TNFα in wild type DCs (Figure 23). In contrast, Zn^{2+} led to an enhanced production of IL-10 (at concentrations 100 µM and 1 mM) and TNFα (100 and 300 µM Zn^{2+}) (Figure 24).

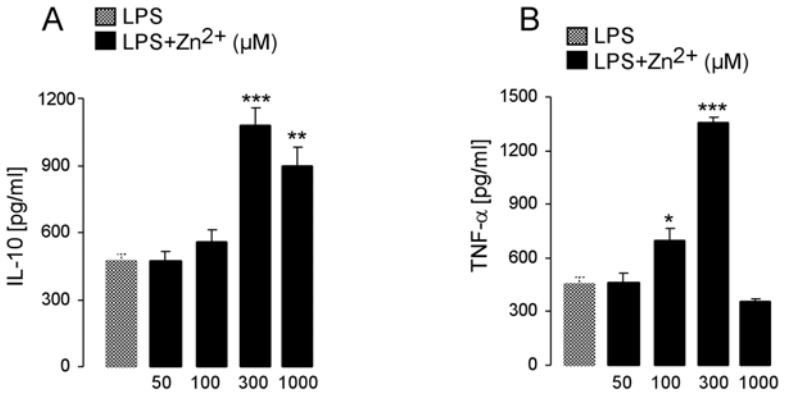

Fig. 24

Figure 24: Effect of Zn^{2+} on cytokine secretion by LPS- stimulated DCs

Arithmetic means (n = 4 each) of IL-10 (**A**) and TNFα (**B**) secretion in LPS-stimulated (100 ng/ml, 24h) untreated (grey bars) or treated with Zn^{2+} (50-1000 μM, black bars. * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) indicate significant difference from LPS-stimulated DCs, ANOVA.

5. DICUSSION

The present study has been performed to explore whether DC maturation and survival are affected by different nutrients, such as thymoquinone, GA, Zn^{2+}, thymol and xanthohumol. GA was a powerful agent inducing DC maturation. On the other hand, thymoquinone, xanthohumol and thymol impaired secretion of several cytokines from LPS-stimulated DCs and thymoquinone inhibited the maturation of DCs. Moreover, Zn^{2+}, thymol, xanthohumol and thymoquinone led to enhanced DC apoptosis that could suppress the immune response, an effect which, at least in theory, may contribute to the anti-inflammatory action of these nutrients (Cho et al 2008e).

Triggering of DC apoptosis by Zn^{2+}, xanthohumol and thymol was mediated by acid sphingomyelinase and subsequent formation of ceramide. Accordingly, genetic knockout of acid sphingomyelinase abrogates the proapoptotic effect of these nutrients. In the present experiments, the stimulation of apoptosis by the nutrients was indeed mimicked by administration of C2-ceramide. Ceramide has been shown to participate in the stimulation of cell death in a variety of cells including T-lymphocytes (Gulbins et al 1997), hepatocytes (Lang et al 2007), erythrocytes (Bentzen et al 2007; Lang et al 2006; Nicolay et al 2006) and pancreatic beta cells (Newsholme et al 2007).

In human DCs it was shown that ceramide can induce cell death in the absence of serum and that pharmacological inhibition of neutral/alkaline ceramidases, which leads to accumulation of C2 ceramide, sensitizes DCs to ceramide-induced cell death (Franchi et al 2006a; Franchi et al 2006b). Antigen uptake and presentation by DCs could be inhibited by exogenously added or endogenously produced ceramides (Sallusto et al 1996). Sphingosine-1-phosphate (S1P), which is generated from ceramide by the consecutive actions of ceramidase and sphingosine kinase was found to be a counterplayer of ceramide which can potently induce cell proliferation (Huwiler and Pfeilschifter 2006). S1P was shown to mediate migration of mature murine DCs (Czeloth et al 2005). Moreover, inhibiting sphingosine kinase suppressed a Th1 polarization via the inhibition of immunostimulatory activity in murine DCs (Jung et al 2007). Thus, a balance between ceramide and S1P may be decisive for DC responsiveness.

Compelling evidence supports the importance of DC survival in the control of immune responses. Mature DCs are short-lived cells both *in vitro* and *in vivo*. The short life span of these cells could represent an important mechanism controlling the normal immune response and ensuring adequate space for the constant influx of fresh DCs loaded with different antigens (Josien et al 2000b; Seifarth et al 2008). DCs with an increased lifespan can induce

stronger immune responses and even autoimmunity (Josien et al 2000a; Wang et al 1999). On the other hand, premature apoptosis in DCs could impair the nascent T cell-dependent response and thus weaken the defense against infectious disease. DCs appear to exhibit mechanisms that counterbalance apoptotic stimuli that otherwise efficiently induce apoptosis in macrophages. Thus, mature DCs are relatively resistant to the proapoptotic action of TNFα (Leverkus et al 2000; Lundqvist et al 2002a) and CD95-mediated apoptosis (Ashany et al 1999b). Their resistance is associated with the up-regulation of FLIP {Ashany, 1999 54 /id} and Bcl-x_L (Lundqvist et al 2002b).

5.1. Thymoquinone

The present study reveals a completely novel effect of thymoquinone. The LPS-induced stimulation of CD86, CD54, CD40 and MHC class II expression in DCs is significantly blunted at higher concentrations of thymoquinone. A decrease of CD86 and CD40 is typical for a tolerogenic DC phenotype (Chorny et al 2006b). Accordingly, thymoquinone is expected to blunt the immune response to LPS.

Similar to LPS-induced maturation, the LPS-induced release of IL-10, IL-12p70 and TNFα is blunted in the presence of thymoquinone. For a statistically significant blunting of cytokine release thymoquinone concentrations of as little as 1 µM are required. Thus, it appears that the cytokine release is particularly sensitive to thymoquinone.

The exposure of LPS- treated DCs to thymoquinone led to an enhanced phosphatidylserine scrambling. The apoptotic potency of thymoquinone has previously been shown in tumor cells (Gali-Muhtasib et al 2004c; Roepke et al 2007a; Rooney and Ryan 2005d; Shoieb et al 2003b) and is assumed to account for the anticarcinogenic potency of the drug (Aggarwal et al 2008c; Ali and Blunden 2003c; Gali-Muhtasib, Roessner, and Schneider-Stock 2006; Gali-Muhtasib et al 2008a; Mohamed, Shoker, Bendjelloul, Mare, Alzrigh, Benghuzzi, and Desin 2003; Salem 2005d). The present data clarify that the thymoquinone induced apoptosis may similarly contribute to its anti-inflammatory potential.

In conclusion, this present study reveals that thymoquinone inhibits LPS- induced DC maturation, cytokine formation and survival. The observations provide mechanisms most likely contributing to the known anti-inflammatory effect of this medically valuable nutrient.

5.2. Gum Arabic

The present study reveals a completely novel effect of GA, i.e. its ability to modify the maturation of dendritic cells (DCs). Similar to bacterial LPS, GA exposure leads to the upregulation of several maturation markers, such as CD86, CD54, CD40 and MHC II. Treatment with GA further stimulates the formation of IL-6, IL-10 and IL-12p70 as well as

TNFα. The formation of those cytokines can also be stimulated by LPS. Nevertheless, the effects of LPS and GA are markedly different. Whereas GA has a particularly strong effect on IL-10 formation, LPS is by far more effective on IL-6 and IL-12p70 formation. Notably, IL-10 is a negative feedback inhibitor of exuberant T cell responses (Li and Flavell 2008a). High amounts of IL-10 may lead to tolerance since IL-10 can suppress T cells or lead to regulatory T cell differentiation; IL-10 can also act on DCs to decrease their function or make them tolerogenic (Steinman and Nussenzweig 2002a). Thus, GA may, unlike LPS, exert anti-inflammatory effects.

The *in vivo* effects of GA on the immune system may involve additional mechanisms beyond its direct effect on DCs. GA may influence the immune response by decreasing the plasma concentration of $1,25(OH)_2D_3$ (Nasir et al 2008a), a hormone produced in activated DCs and macrophages (Fritsche et al 2003; Hewison et al 2003a; Hewison et al 2003b). $1,25(OH)_2D_3$ can act directly on T cells, but antigen-presenting cells, and in particular DCs, appear to be primary targets for its tolerogenic properties (Adorini et al 2004). $1,25(OH)_2D_3$ inhibits the differentiation and maturation of DCs as well as their capacity to secrete the Th1-polarizing cytokine IL-12 (Gauzzi et al 2005; Lyakh et al 2005; Penna et al 2007; van Etten and Mathieu 2005). GA may further lead to the formation of short-chain fatty acids (Bliss 2004b) with subsequent increase of serum butyrate concentrations (Matsumoto et al 2006b). Butyrate has in turn been shown to inhibit the functional differentiation of DCs (Millard et al 2002; Saemann et al 2002; Wang et al 2008a).

Our data demonstrates that stimulation by GA resulted in an increased activity of extracellular signal-regulated kinase ERK1/2. Enhanced activity of this MAPK fits well the observed high-level secretion of IL-10 by GA-stimulated DCs, since it was reported that high-level of ERK phosphorylation is associated with IL-10 production (Xia et al 2005).

In conclusion, this present study reveals that GA is a powerful stimulator of DC maturation and cytokine formation. Thus, GA is a novel nutrient with immunoregulatory potency.

5.3. Zn^{2+}

The present study unravels novel effects of Zn^{2+}, i.e. the stimulation of ceramide production in and suicidal death of DCs. The ability of Zn^{2+} to stimulate the sphingomyelinase has been shown before in other cell types (Tabas 1999a).

Zn^{2+} is necessary for the normal function of the immune system. However a variety of *in vivo* and *in vitro* effects of Zn^{2+} on immune cells mainly depend on the Zn^{2+} concentration (Ibs and Rink 2003a; Murakami and Hirano 2008b). The concentrations of Zn^{2+} required to elicit the effects described in the present study are comparable with Zn^{2+} concentrations in blood,

which approaches 0.3 mg/l corresponding to approximately 50 µM (Massadeh et al 2009; VALLEE and GIBSON 1948). However, most of blood Zn^{2+} is bound to protein (Blindauer et al 2009) and thus, the plasma concentration of free Zn^{2+} is lower than that of total Zn^{2+}. Intracellular Zn^{2+} concentrations were assessed to be in the femtomol/l range, indicating that the cells exert tight control over cytosolic Zn^{2+} concentrations (Outten and O'Halloran 2001).

Zn^{2+} can lead to apoptosis in several cell systems. Thus, Zn^{2+} influx through Ca^{2+}-permeable AMPA/kainate (Ca-A/K) channels triggers reactive oxygen species generation and is potently neurotoxic (Weiss and Sensi 2000). Accumulation of Zn^{2+} in postsynaptic neurons may contribute to the selective neuronal loss that is associated with certain acute conditions, including epilepsy and transient global ischaemia (Weiss et al 2000). Zn^{2+} was also shown to induce apopotitic death in lymphocytes, including T and B cells (Ibs and Rink 2003c; Telford and Fraker 1995).

Exposure of mouse DCs to LPS leads to a decrease in the intracellular Zn^{2+} concentration (Kitamura et al 2006b). Moreover, artificially depleting the intracellular Zn^{2+} using a Zn^{2+} chelator triggers DC maturation. On the other hand, elevating Zn^{2+} levels suppresses the ability of DCs to respond to LPS. Zn^{2+} may suppress the surface expression of MHC class II because Zn^{2+} is required for the endocytosis of MHC class II expressed on the plasma membrane and Zn^{2+} inhibits MHC class II vesicle trafficking to the plasma membrane from the perinuclear region (Kitamura et al 2006a). Besides that higher concentrations of Zn^{2+} can induce apoptosis of DCs. And thus, by lowering intracellular Zn^{2+} level LPS may also contribute to an enhanced survival of DCs. Zn^{2+} was also reported to abrogate the LPS-induced release of TNF-alpha and IL-1β in monocytes, an effect mediating by increase of intracellular cGMP levels (von Issendorff and Cheshnovsky 2005). High concentrations of Zn^{2+} thus predispose to bacterial and viral infections (Fischer-Walker and Black 2004; Fraker and King 2004; Prasad 1998b), which may, however, not be primarily due to the role of Zn^{2+} in DC function and survival, but may at least partially result from deranged function of lymphocytes (Hosea et al 2003a; Prasad 2000b).

In conclusion, the present study reveals that Zn^{2+} stimulates ceramide formation and apoptotic death of DCs. The effect is expected to affect the immune response.

5.4. Xanthohumol

According to the present study, xanthohumol stimulates the acid sphingomyelinase in DCs leading to ceramide formation, caspase activation and stimulation of suicidal cell death.

Xanthohumol has previously been shown to trigger apoptosis in a wide variety of cells including adipocytes (Yang et al 2007; Yang et al 2008), preadipocytes (Mendes et al 2008),

leukemia cells (Dell'Eva et al 2007a; Diller et al 2005a; Harikumar et al 2009b; Lust et al 2005b), breast cancer cells (Gerhauser et al 2002b; Guerreiro et al 2007b; Monteiro et al 2007b; Monteiro et al 2008b; Vanhoecke et al 2005b), prostate cancer (Colgate et al 2007b), hepatocellular carcinoma cells (Ho et al 2008b), colon cancer cells (Pan et al 2005b), Bcr/Abl-transformed cells (Monteghirfo et al 2008a), fibrosarcoma (Goto et al 2005b), Kaposi's sarcoma (Larghero et al 2007b). An effect of xanthohumol on DC survival has never been reported.

Xanthohumol is partially effective through activation of the death receptor- and mitochondrial pathway (Pan et al 2005a), nuclear factor-kappaB (NFκB) and p53 modulation (Albini and Pfeffer 2006; Colgate et al 2007a; Harikumar et al 2009a; Monteghirfo et al 2008c). Moreover, xanthohumol has been shown to inhibit diacylglycerol acyltransferase (Goto et al 2005a) and to upregulate the function of the E-cadherin/catenin complex (Vanhoecke et al 2005a). Xanthohumol may increase the cellular content of reactive oxidant species but at the same time may exert antioxidant activity (Vogel and Heilmann 2008). Moreover, xanthohumol has been shown to upregulate the detoxification enzyme NADPH-quinone oxidoreductase (Dietz et al 2005). An involvement of sphingomyelinase and/or ceramide in the effects of xanthohumol has not been published.

In contrast to its proapoptotic effect on nucleated cells, xanthohumol protects erythrocytes against suicidal cell death (Quadri et al 2009), which is, similar to apoptosis of nucleated cells, stimulated by stimulation of sphingomyelinase and subsequent formation of ceramide (Brand et al 2008b; Lang et al 2008a; Sopjani et al 2008a; Sopjani et al 2008c; Wang et al 2008b). The mechanisms accounting for the differences between nucleated cells and erythrocytes during suicidal death remain to be elucidated. Clearly, the suicidal death of erythrocytes lacking nuclei is not expected to be modified by transcription factors such as NFκB (Lang, Gulbins, Lerche, Huber, Kempe, and Foller 2008a).

The xanthohumol-induced DC apoptosis does, of course, not rule out additional mechanisms contributing to the anti-inflammatory effect of this nutrient.

In conclusion, xanthohumol stimulates ceramide formation and apoptotic death of DCs. The effect is expected to affect the immune response.

5.5. Thymol

Our study reveals that thymol stimulates the acid sphingomyelinase in DCs with subsequent formation of ceramide, caspase activation, down- regulating the expression of anti- apoptotic Bcl-2 and Bcl-xL proteins and finally triggering of suicidal cell death.

To the best of our knowledge, an effect of thymol on sphingomyelinase or on ceramide formation has never been shown before. Thymol has been shown to exert some antioxidant activity (Undeger et al 2009a) and to decrease the cytosolic Ca^{2+} activity (Suzuki et al 1987). Both effects would not be expected to induce cell death. In contrast, stimulation of sphingomyelinase and subsequent formation of ceramide are well known to induce apoptosis in a variety of cell types (Carpinteiro et al 2008a; Grassme et al 2008b; Jana et al 2009a; Lang, Gulbins, Lerche, Huber, Kempe, and Foller 2008a; Perrotta et al 2008b; Smith and Schuchman 2008b).

The observation, that thymol stimulates caspases and suicidal cell death, is similarly novel. In erythrocytes thymol even protects against suicidal cell death (Mahmud, Mauro, Föller, and Lang 2009), which is induced by stimulation of sphingomyelinase and subsequent formation of ceramide (Brand et al 2008a; Lang, Gulbins, Lerche, Huber, Kempe, and Foller 2008a; Sopjani, Foller, Dreischer, and Lang 2008a; Sopjani et al 2008b; Wang, Mahmud, Foller, Biswas, Lang, Bohn, Gotz, and Lang 2008b). The present oberservations do not disclose the mechanisms underlying opposite effects of thymol on cell survival of erythrocytes and DCs. Clearly, the regulation of ceramide formation is distinct in DCs and erythrocytes. Moreover, the signaling of suicidal death is different between erythrocytes and nucleated cells, as erythrocytes lack mitochondria and nuclei, key players in apoptosis (Lang, Gulbins, Lerche, Huber, Kempe, and Foller 2008a).

The thymol induced apoptosis is expected to weaken the immune response, an effect which, at least in theory, may limit its use in the treatment of infectious disease. However, the effect on pathogens may be more pronounced and thymol may thus favourably influence the course of infectious disease (Burt 2004b; Cervenka et al 2008a; Corbo et al 2008b; Lee and Jin 2008a; Razzaghi-Abyaneh et al 2008a). Nevertheless, the present observations provide a caveat on the use of thymol in infectious disease. On the other hand, the thymol induced apoptosis may exert some antiinflammatory action.

In conclusion, thymol stimulates ceramide formation and apoptotic death of dendritic cells. The effect is expected to affect the immune response.

6. REFERENCES

References

Aderem,A., Underhill,D.M., 1999. Mechanisms of phagocytosis in macrophages. Annu. Rev. Immunol. 17, 593-623.

Adler,H.S., Steinbrink,K., 2007. Tolerogenic dendritic cells in health and disease: friend and foe! Eur J Dermatol. 17, 476-491.

Adorini,L., Giarratana,N., Penna,G., 2004. Pharmacological induction of tolerogenic dendritic cells and regulatory T cells. Semin. Immunol 16, 127-134.

Aggarwal,B.B., Kunnumakkara,A.B., Harikumar,K.B., Tharakan,S.T., Sung,B., Anand,P., 2008b. Potential of spice-derived phytochemicals for cancer prevention. Planta Med 74, 1560-1569.

Akira,S., Takeda,K., 2004. Toll-like receptor signalling. Nat. Rev. Immunol. 4, 499-511.

Al Majed,A.A., Mostafa,A.M., Al Rikabi,A.C., Al Shabanah,O.A., 2002a. Protective effects of oral arabic gum administration on gentamicin-induced nephrotoxicity in rats. Pharmacol. Res. 46, 445-451.

Alaniz,R.C., Sandall,S., Thomas,E.K., Wilson,C.B., 2004. Increased dendritic cell numbers impair protective immunity to intracellular bacteria despite augmenting antigen-specific CD8+ T lymphocyte responses. J. Immunol. 172, 3725-3735.

Albini,A., Pfeffer,U., 2006. A new tumor suppressor gene: invasion, metastasis, and angiogenesis as potential key targets. J Natl Cancer Inst. 98, 800-801.

Ali,B.H., Blunden,G., 2003b. Pharmacological and toxicological properties of Nigella sativa. Phytother. Res 17, 299-305.

An,H., Yu,Y., Zhang,M., Xu,H., Qi,R., Yan,X., Liu,S., Wang,W., Guo,Z., Guo,J., Qin,Z., Cao,X., 2002. Involvement of ERK, p38 and NF-kappaB signal transduction in regulation of TLR2, TLR4 and TLR9 gene expression induced by lipopolysaccharide in mouse dendritic cells. Immunology 106, 38-45.

Anderson,D.M., 1986. Evidence for the safety of gum arabic (Acacia senegal (L.) Willd.) as a food additive--a brief review. Food Addit. Contam 3, 225-230.

Ashany,D., Savir,A., Bhardwaj,N., Elkon,K.B., 1999a. Dendritic cells are resistant to apoptosis through the Fas (CD95/APO-1) pathway. J. Immunol. 163, 5303-5311.

Ashkenazi,A., Dixit,V.M., 1998. Death receptors: signaling and modulation. Science 281, 1305-1308.

Aydin,S., Basaran,A.A., Basaran,N., 2005. Modulating effects of thyme and its major ingredients on oxidative DNA damage in human lymphocytes. J Agric. Food Chem. 53, 1299-1305.

Badary,O.A., Abd-Ellah,M.F., El Mahdy,M.A., Salama,S.A., Hamada,F.M., 2007. Anticlastogenic activity of thymoquinone against benzo(a)pyrene in mice. Food Chem. Toxicol 45, 88-92.

Ballou,L.R., Laulederkind,S.J., Rosloniec,E.F., Raghow,R., 1996. Ceramide signalling and the immune response. Biochim. Biophys. Acta 1301, 273-287.

Banchereau,J., Briere,F., Caux,C., Davoust,J., Lebecque,S., Liu,Y.J., Pulendran,B., Palucka,K., 2000b. Immunobiology of dendritic cells. Annu. Rev. Immunol 18, 767-811.

Bentzen,P.J., Lang,E., Lang,F., 2007. Curcumin induced suicidal erythrocyte death. Cell Physiol Biochem. 19, 153-164.

Berger,M.M., Shenkin,A., 2007. Trace element requirements in critically ill burned patients. J Trace Elem. Med Biol 21 Suppl 1, 44-48.

Bjorck,P., 2001. Isolation and characterization of plasmacytoid dendritic cells from Flt3 ligand and granulocyte-macrophage colony-stimulating factor-treated mice. Blood 98, 3520-3526.

Blanchard,C., Rothenberg,M.E., 2009. Biology of the eosinophil. Adv. Immunol. 101, 81-121.

Blanco,P., Palucka,A.K., Pascual,V., Banchereau,J., 2008. Dendritic cells and cytokines in human inflammatory and autoimmune diseases. Cytokine Growth Factor Rev. 19, 41-52.

Blindauer,C.A., Harvey,I., Bunyan,K.E., Stewart,A.J., Sleep,D., Harrison,D.J., Berezenko,S., Sadler,P.J., 2009. Structure, properties, and engineering of the major zinc binding site on human albumin. J Biol Chem.

Bliss,D.Z., 2004b. Dietary fiber in conservative management of chronic renal failure. Pediatr. Nephrol. 19, 1069-1070.

Bliss,D.Z., Stein,T.P., Schleifer,C.R., Settle,R.G., 1996. Supplementation with gum arabic fiber increases fecal nitrogen excretion and lowers serum urea nitrogen concentration in chronic renal failure patients consuming a low-protein diet. Am. J. Clin. Nutr. 63, 392-398.

Boatright,K.M., Salvesen,G.S., 2003a. Mechanisms of caspase activation. Curr. Opin. Cell Biol. 15, 725-731.

Brand,V., Koka,S., Lang,C., Jendrossek,V., Huber,S.M., Gulbins,E., Lang,F., 2008a. Influence of amitriptyline on eryptosis, parasitemia and survival of Plasmodium berghei-infected mice. Cell Physiol Biochem 22, 405-412.

Brown,K.H., Lopez,d.R., Arsenault,J.E., Peerson,J.M., Penny,M.E., 2007. Comparison of the effects of zinc delivered in a fortified food or a liquid supplement on the growth,

morbidity, and plasma zinc concentrations of young Peruvian children. Am J Clin Nutr. 85, 538-547.

Burt,S., 2004b. Essential oils: their antibacterial properties and potential applications in foods--a review. Int J Food Microbiol 94, 223-253.

Carpinteiro,A., Dumitru,C., Schenck,M., Gulbins,E., 2008b. Ceramide-induced cell death in malignant cells. Cancer Lett. 264, 1-10.

Cella,M., Engering,A., Pinet,V., Pieters,J., Lanzavecchia,A., 1997. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. Nature 388, 782-787.

Cerovic,V., McDonald,V., Nassar,M.A., Paulin,S.M., Macpherson,G.G., Milling,S.W., 2009. New insights into the roles of dendritic cells in intestinal immunity and tolerance. Int. Rev. Cell Mol Biol 272, 33-105.

Cervenka,L., Peskova,I., Pejchalova,M., Vytrasova,J., 2008b. Inhibition of Arcobacter butzleri, Arcobacter cryaerophilus, and Arcobacter skirrowii by plant oil aromatics. J Food Prot. 71, 165-169.

Chen,M., Quintans,J., Fuks,Z., Thompson,C., Kufe,D.W., Weichselbaum,R.R., 1995. Suppression of Bcl-2 messenger RNA production may mediate apoptosis after ionizing radiation, tumor necrosis factor alpha, and ceramide. Cancer Res. 55, 991-994.

Cheng,E.H., Kirsch,D.G., Clem,R.J., Ravi,R., Kastan,M.B., Bedi,A., Ueno,K., Hardwick,J.M., 1997. Conversion of Bcl-2 to a Bax-like death effector by caspases. Science 278, 1966-1968.

Cho,Y.C., Kim,H.J., Kim,Y.J., Lee,K.Y., Choi,H.J., Lee,I.S., Kang,B.Y., 2008d. Differential anti-inflammatory pathway by xanthohumol in IFN-gamma and LPS-activated macrophages. Int Immunopharmacol. 8, 567-573.

Choi,J.M., Kim,H.J., Lee,K.Y., Choi,H.J., Lee,I.S., Kang,B.Y., 2009b. Increased IL-2 production in T cells by xanthohumol through enhanced NF-AT and AP-1 activity. Int Immunopharmacol. 9, 103-107.

Chorny,A., Gonzalez-Rey,E., Delgado,M., 2006a. Regulation of dendritic cell differentiation by vasoactive intestinal peptide: therapeutic applications on autoimmunity and transplantation. Ann. N. Y. Acad Sci 1088, 187-194.

Chowdhury,I., Tharakan,B., Bhat,G.K., 2008. Caspases - an update. Comp Biochem. Physiol B Biochem. Mol. Biol. 151, 10-27.

Coban,C., Ishii,K.J., Kawai,T., Hemmi,H., Sato,S., Uematsu,S., Yamamoto,M., Takeuchi,O., Itagaki,S., Kumar,N., Horii,T., Akira,S., 2005. Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin. J. Exp. Med. 201, 19-25.

Coles,C.L., Bose,A., Moses,P.D., Mathew,L., Agarwal,I., Mammen,T., Santosham,M., 2007. Infectious etiology modifies the treatment effect of zinc in severe pneumonia. Am J Clin Nutr. 86, 397-403.

Colgate,E.C., Miranda,C.L., Stevens,J.F., Bray,T.M., Ho,E., 2007b. Xanthohumol, a prenylflavonoid derived from hops induces apoptosis and inhibits NF-kappaB activation in prostate epithelial cells. Cancer Lett 246, 201-209.

Colonna,M., Trinchieri,G., Liu,Y.J., 2004. Plasmacytoid dendritic cells in immunity. Nat. Immunol. 5, 1219-1226.

Corbo,M.R., Speranza,B., Filippone,A., Granatiero,S., Conte,A., Sinigaglia,M., Del Nobile,M.A., 2008a. Study on the synergic effect of natural compounds on the microbial quality decay of packed fish hamburger. Int J Food Microbiol 127, 261-267.

Corthay,A., 2006. A three-cell model for activation of naive T helper cells. Scand. J. Immunol. 64, 93-96.

Cruz,P.D., Jr., Bergstresser,P.R., 1990. Antigen processing and presentation by epidermal Langerhans cells. Induction of immunity or unresponsiveness. Dermatol. Clin. 8, 633-647.

Cuevas,L.E., Koyanagi,A., 2005. Zinc and infection: a review. Ann. Trop. Paediatr. 25, 149-160.

Czeloth,N., Bernhardt,G., Hofmann,F., Genth,H., Forster,R., 2005. Sphingosine-1-phosphate mediates migration of mature dendritic cells. J. Immunol. 175, 2960-2967.

Deckwer,W.D., Dill,B., Eisenbrand,E., Bornscheuer,U., Pühler,A., Heiker,F.R., Kirschning,A., Schreier,P., Fugmann,B., Pohnert,G., Gamse,T., Hulpke,H., 2006. Römpp Online. Georg-Thieme-Verlag.

Delcenserie,V., Martel,D., Lamoureux,M., Amiot,J., Boutin,Y., Roy,D., 2008. Immunomodulatory effects of probiotics in the intestinal tract. Curr. Issues Mol. Biol. 10, 37-54.

Dell'Eva,R., Ambrosini,C., Vannini,N., Piaggio,G., Albini,A., Ferrari,N., 2007a. AKT/NF-kappaB inhibitor xanthohumol targets cell growth and angiogenesis in hematologic malignancies. Cancer 110, 2007-2011.

Delves,P.J., Roitt,I.M., 2000. The immune system. First of two parts. N. Engl. J. Med. 343, 37-49.

den Haan,J.M., Lehar,S.M., Bevan,M.J., 2000. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. J. Exp. Med. 192, 1685-1696.

Dent,A.L., Kaplan,M.H., 2008. T cell regulation of hematopoiesis. Front Biosci. 13, 6229-6236.

Dhodapkar,M.V., Steinman,R.M., Krasovsky,J., Munz,C., Bhardwaj,N., 2001. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. J. Exp. Med. 193, 233-238.

Dietz,B.M., Kang,Y.H., Liu,G., Eggler,A.L., Yao,P., Chadwick,L.R., Pauli,G.F., Farnsworth,N.R., Mesecar,A.D., van Breemen,R.B., Bolton,J.L., 2005. Xanthohumol isolated from Humulus lupulus Inhibits menadione-induced DNA damage through induction of quinone reductase. Chem. Res Toxicol 18, 1296-1305.

Diller,R.A., Riepl,H.M., Rose,O., Frias,C., Henze,G., Prokop,A., 2005a. Synthesis of demethylxanthohumol, a new potent apoptosis-inducing agent from hops. Chem. Biodivers. 2, 1331-1337.

Dressler,K.A., Kan,C.C., Kolesnick,R.N., 1991. Sphingomyelin synthesis is involved in adherence during macrophage differentiation of HL-60 cells. J. Biol. Chem. 266, 11522-11527.

Edelman,S.M., Kasper,D.L., 2008. Symbiotic commensal bacteria direct maturation of the host immune system. Curr. Opin Gastroenterol. 24, 720-724.

El Gazzar,M., El Mezayen,R., Marecki,J.C., Nicolls,M.R., Canastar,A., Dreskin,S.C., 2006a. Anti-inflammatory effect of thymoquinone in a mouse model of allergic lung inflammation. Int Immunopharmacol. 6, 1135-1142.

El Gazzar,M., El Mezayen,R., Nicolls,M.R., Marecki,J.C., Dreskin,S.C., 2006c. Downregulation of leukotriene biosynthesis by thymoquinone attenuates airway inflammation in a mouse model of allergic asthma. Biochim. Biophys. Acta 1760, 1088-1095.

El Gazzar,M.A., 2007. Thymoquinone suppressses in vitro production of IL-5 and IL-13 by mast cells in response to lipopolysaccharide stimulation. Inflamm. Res 56, 345-351.

El Gazzar,M.A., El Mezayen,R., Nicolls,M.R., Dreskin,S.C., 2007. Thymoquinone attenuates proinflammatory responses in lipopolysaccharide-activated mast cells by modulating NF-kappaB nuclear transactivation. Biochim. Biophys. Acta 1770, 556-564.

El Mahdy,M.A., Zhu,Q., Wang,Q.E., Wani,G., Wani,A.A., 2005. Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. Int J Cancer 117, 409-417.

El Mezayen,R., El Gazzar,M., Nicolls,M.R., Marecki,J.C., Dreskin,S.C., Nomiyama,H., 2006. Effect of thymoquinone on cyclooxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation. Immunol Lett 106, 72-81.

Faria,A.M., Weiner,H.L., 2005. Oral tolerance. Immunol. Rev. 206, 232-259.

Fernandez,N.C., Lozier,A., Flament,C., Ricciardi-Castagnoli,P., Bellet,D., Suter,M., Perricaudet,M., Tursz,T., Maraskovsky,E., Zitvogel,L., 1999. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. Nat. Med. 5, 405-411.

Fischer-Walker,C., Black,R.E., 2004. Zinc and the risk for infectious disease. Annu. Rev. Nutr. 24, 255-275.

Fraker,P.J., King,L.E., 2004. Reprogramming of the immune system during zinc deficiency. Annu. Rev. Nutr. 24, 277-298.

Franchi,L., Malisan,F., Tomassini,B., Testi,R., 2006a. Ceramide catabolism critically controls survival of human dendritic cells. J. Leukoc. Biol. 79, 166-172.

Fritsche,J., Mondal,K., Ehrnsperger,A., Andreesen,R., Kreutz,M., 2003. Regulation of 25-hydroxyvitamin D3-1 alpha-hydroxylase and production of 1 alpha,25-dihydroxyvitamin D3 by human dendritic cells. Blood 102, 3314-3316.

Gad,M., Claesson,M.H., Pedersen,A.E., 2003. Dendritic cells in peripheral tolerance and immunity. APMIS 111, 766-775.

Gali-Muhtasib,H., Diab-Assaf,M., Boltze,C., Al Hmaira,J., Hartig,R., Roessner,A., Schneider-Stock,R., 2004a. Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a p53-dependent mechanism. Int J Oncol. 25, 857-866.

Gali-Muhtasib,H., Ocker,M., Kuester,D., Krueger,S., El Hajj,Z., Diestel,A., Evert,M., El Najjar,N., Peters,B., Jurjus,A., Roessner,A., Schneider-Stock,R., 2008a. Thymoquinone reduces mouse colon tumor cell invasion and inhibits tumor growth in murine colon cancer models. J Cell Mol Med 12, 330-342.

Gali-Muhtasib,H., Roessner,A., Schneider-Stock,R., 2006. Thymoquinone: a promising anti-cancer drug from natural sources. Int J Biochem Cell Biol 38, 1249-1253.

Gali-Muhtasib,H.U., Abou Kheir,W.G., Kheir,L.A., Darwiche,N., Crooks,P.A., 2004c. Molecular pathway for thymoquinone-induced cell-cycle arrest and apoptosis in neoplastic keratinocytes. Anticancer Drugs 15, 389-399.

Galli,S.J., Grimbaldeston,M., Tsai,M., 2008. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. Nat. Rev. Immunol. 8, 478-486.

Gao,X., Deeb,D., Liu,Y., Gautam,S., Dulchavsky,S.A., Gautam,S.C., 2009. Immunomodulatory activity of xanthohumol: inhibition of T cell proliferation, cell-mediated cytotoxicity and Th1 cytokine production through suppression of NF-kappaB. Immunopharmacol. Immunotoxicol.

Gauzzi,M.C., Purificato,C., Donato,K., Jin,Y., Wang,L., Daniel,K.C., Maghazachi,A.A., Belardelli,F., Adorini,L., Gessani,S., 2005. Suppressive effect of 1alpha,25-dihydroxyvitamin D3 on type I IFN-mediated monocyte differentiation into dendritic cells: impairment of functional activities and chemotaxis. J Immunol 174, 270-276.

Gelin,C., Sloma,I., Charron,D., Mooney,N., 2009. Regulation of MHC II and CD1 antigen presentation: from ubiquity to security. J Leukoc. Biol 85, 215-224.

Georgieff,M.K., 2007. Nutrition and the developing brain: nutrient priorities and measurement. Am J Clin Nutr. 85, 614S-620S.

Gerhauser,C., 2005. Beer constituents as potential cancer chemopreventive agents. Eur J Cancer 41, 1941-1954.

Gerhauser,C., Alt,A., Heiss,E., Gamal-Eldeen,A., Klimo,K., Knauft,J., Neumann,I., Scherf,H.R., Frank,N., Bartsch,H., Becker,H., 2002a. Cancer chemopreventive activity of Xanthohumol, a natural product derived from hop. Mol Cancer Ther. 1, 959-969.

Gerloni,M., Lo,D., Zanetti,M., 1998. DNA immunization in relB-deficient mice discloses a role for dendritic cells in IgM-->IgG1 switch in vivo. Eur. J. Immunol. 28, 516-524.

Goto,K., Asai,T., Hara,S., Namatame,I., Tomoda,H., Ikemoto,M., Oku,N., 2005b. Enhanced antitumor activity of xanthohumol, a diacylglycerol acyltransferase inhibitor, under hypoxia. Cancer Lett 219, 215-222.

Gradzka,I., 2006. [Mechanisms and regulation of the programmed cell death]. Postepy Biochem. 52, 157-165.

Grassme,H., Becker,K.A., Zhang,Y., Gulbins,E., 2008b. Ceramide in bacterial infections and cystic fibrosis. Biol Chem. 389, 1371-1379.

Green,D.R., Reed,J.C., 1998a. Mitochondria and apoptosis. Science 281, 1309-1312.

Guerreiro,S., Monteiro,R., Martins,M.J., Calhau,C., Azevedo,I., Soares,R., 2007b. Distinct modulation of alkaline phosphatase isoenzymes by 17beta-estradiol and xanthohumol in breast cancer MCF-7 cells. Clin Biochem 40, 268-273.

Gulbins,E., Szabo,I., Baltzer,K., Lang,F., 1997. Ceramide-induced inhibition of T lymphocyte voltage-gated potassium channel is mediated by tyrosine kinases. Proc. Natl. Acad. Sci. U. S. A 94, 7661-7666.

Guo,J., Nikolic,D., Chadwick,L.R., Pauli,G.F., van Breemen,R.B., 2006. Identification of human hepatic cytochrome P450 enzymes involved in the metabolism of 8-prenylnaringenin and isoxanthohumol from hops (Humulus lupulus L.). Drug Metab Dispos. 34, 1152-1159.

Guo,N., Liu,J., Wu,X., Bi,X., Meng,R., Wang,X., Xiang,H., Deng,X., Yu,L., 2009. Antifungal activity of thymol against clinical isolates of fluconazole-sensitive and -resistant Candida albicans. J. Med. Microbiol. 58, 1074-1079.

Haase,C., Jorgensen,T.N., Michelsen,B.K., 2002a. Both exogenous and endogenous interleukin-10 affects the maturation of bone-marrow-derived dendritic cells in vitro and strongly influences T-cell priming in vivo. Immunology 107, 489-499.

Haimovitz-Friedman,A., Kan,C.C., Ehleiter,D., Persaud,R.S., McLoughlin,M., Fuks,Z., Kolesnick,R.N., 1994. Ionizing radiation acts on cellular membranes to generate ceramide and initiate apoptosis. J. Exp. Med. 180, 525-535.

Haldar,S., Jena,N., Croce,C.M., 1994. Antiapoptosis potential of bcl-2 oncogene by dephosphorylation. Biochem. Cell Biol. 72, 455-462.

Haldar,S., Jena,N., Croce,C.M., 1995. Inactivation of Bcl-2 by phosphorylation. Proc. Natl. Acad. Sci. U. S. A 92, 4507-4511.

Hambidge,K.M., 2006. Zinc and pneumonia. Am J Clin Nutr. 83, 991-992.

Hamerman,J.A., Ogasawara,K., Lanier,L.L., 2005. NK cells in innate immunity. Curr. Opin. Immunol. 17, 29-35.

Hannun,Y.A., Luberto,C., Argraves,K.M., 2001. Enzymes of sphingolipid metabolism: from modular to integrative signaling. Biochemistry 40, 4893-4903.

Hannun,Y.A., Obeid,L.M., 1995. Ceramide: an intracellular signal for apoptosis. Trends Biochem. Sci. 20, 73-77.

Harikumar,K.B., Kunnumakkara,A.B., Ahn,K.S., Anand,P., Krishnan,S., Guha,S., Aggarwal,B.B., 2009a. Modification of the cysteine residues in IkappaBalpha kinase and NF-kappaB (p65) by xanthohumol leads to suppression of NF-kappaB-regulated gene products and potentiation of apoptosis in leukemia cells. Blood 113, 2003-2013.

Harizi,H., Gualde,N., 2005b. The impact of eicosanoids on the crosstalk between innate and adaptive immunity: the key roles of dendritic cells. Tissue Antigens 65, 507-514.

Hawiger,D., Inaba,K., Dorsett,Y., Guo,M., Mahnke,K., Rivera,M., Ravetch,J.V., Steinman,R.M., Nussenzweig,M.C., 2001b. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J. Exp. Med. 194, 769-779.

Heib,V., Becker,M., Taube,C., Stassen,M., 2008. Advances in the understanding of mast cell function. Br. J. Haematol. 142, 683-694.

Hemmi,H., Takeuchi,O., Kawai,T., Kaisho,T., Sato,S., Sanjo,H., Matsumoto,M., Hoshino,K., Wagner,H., Takeda,K., Akira,S., 2000. A Toll-like receptor recognizes bacterial DNA. Nature 408, 740-745.

Hewison,M., Freeman,L., Hughes,S.V., Evans,K.N., Bland,R., Eliopoulos,A.G., Kilby,M.D., Moss,P.A., Chakraverty,R., 2003a. Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. J Immunol 170, 5382-5390.

Hewison,M., Kantorovich,V., Liker,H.R., Van Herle,A.J., Cohan,P., Zehnder,D., Adams,J.S., 2003b. Vitamin D-mediated hypercalcemia in lymphoma: evidence for hormone production by tumor-adjacent macrophages. J Bone Miner. Res 18, 579-582.

Ho,Y.C., Liu,C.H., Chen,C.N., Duan,K.J., Lin,M.T., 2008a. Inhibitory effects of xanthohumol from hops (Humulus lupulus L.) on human hepatocellular carcinoma cell lines. Phytother. Res 22, 1465-1468.

Hosea,H.J., Rector,E.S., Taylor,C.G., 2003a. Zinc-deficient rats have fewer recent thymic emigrant (CD90+) T lymphocytes in spleen and blood. J Nutr. 133, 4239-4242.

Huwiler,A., Pfeilschifter,J., 2006. Altering the sphingosine-1-phosphate/ceramide balance: a promising approach for tumor therapy. Curr. Pharm. Des 12, 4625-4635.

Ibs,K.H., Rink,L., 2003b. Zinc-altered immune function. J Nutr. 133, 1452S-1456S.

Intelmann,D., Haseleu,G., Hofmann,T., 2009. LC-MS/MS quantitation of hop-derived bitter compounds in beer using the ECHO technique. J Agric. Food Chem. 57, 1172-1182.

Iwasaki,A., 2007. Mucosal dendritic cells. Annu. Rev. Immunol. 25, 381-418.

Iwasaki,A., Medzhitov,R., 2004. Toll-like receptor control of the adaptive immune responses. Nat. Immunol. 5, 987-995.

Jana,A., Hogan,E.L., Pahan,K., 2009a. Ceramide and neurodegeneration: susceptibility of neurons and oligodendrocytes to cell damage and death. J Neurol. Sci 278, 5-15.

Janeway,C.A., Jr., 2001b. How the immune system protects the host from infection. Microbes. Infect. 3, 1167-1171.

Joffre,O., Nolte,M.A., Sporri,R., Reis e Sousa, 2009. Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. Immunol. Rev. 227, 234-247.

Josien,R., Li,H.L., Ingulli,E., Sarma,S., Wong,B.R., Vologodskaia,M., Steinman,R.M., Choi,Y., 2000b. TRANCE, a tumor necrosis factor family member, enhances the longevity and adjuvant properties of dendritic cells in vivo. J. Exp. Med. 191, 495-502.

Jung,I.D., Lee,J.S., Kim,Y.J., Jeong,Y.I., Lee,C.M., Lee,M.G., Ahn,S.C., Park,Y.M., 2007. Sphingosine kinase inhibitor suppresses dendritic cell migration by regulating chemokine receptor expression and impairing p38 mitogen-activated protein kinase. Immunology 121, 533-544.

Kadowaki,N., Antonenko,S., Lau,J.Y., Liu,Y.J., 2000. Natural interferon alpha/beta-producing cells link innate and adaptive immunity. J. Exp. Med. 192, 219-226.

Kelsall,B.L., Rescigno,M., 2004. Mucosal dendritic cells in immunity and inflammation. Nat. Immunol. 5, 1091-1095.

Keukeleire,J.D., Janssens,I., Heyerick,A., Ghekiere,G., Cambie,J., Roldan-Ruiz,I., Bockstaele,E.V., Keukeleire,D.D., 2007. Relevance of organic farming and effect of climatological conditions on the formation of alpha-acids, beta-acids, desmethylxanthohumol, and xanthohumol in hop (Humulus lupulus L.). J Agric. Food Chem. 55, 61-66.

Khader,M., Bresgen,N., Eckl,P.M., 2009. In vitro toxicological properties of thymoquinone. Food Chem. Toxicol 47, 129-133.

Kim,D.O., Lee,C.Y., 2004. Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. Crit Rev. Food Sci Nutr. 44, 253-273.

Kitamura,H., Morikawa,H., Kamon,H., Iguchi,M., Hojyo,S., Fukada,T., Yamashita,S., Kaisho,T., Akira,S., Murakami,M., Hirano,T., 2006b. Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function. Nat. Immunol. 7, 971-977.

Kodama,S., Yamamoto,A., Sato,A., Suzuki,K., Yamashita,T., Kemmei,T., Taga,A., Hayakawa,K., 2007. Enantioseparation of isoxanthohumol in beer by hydroxypropyl-gamma-cyclodextrin-modified micellar electrokinetic chromatography. J Agric. Food Chem. 55, 6547-6552.

Kolesnick,R.N., 1991. Sphingomyelin and derivatives as cellular signals. Prog. Lipid Res. 30, 1-38.

Konig,R., Shen,X., Maroto,R., Denning,T.L., 2002. The role of CD4 in regulating homeostasis of T helper cells. Immunol. Res. 25, 115-130.

Korsmeyer,S.J., 1995. Regulators of cell death. Trends Genet. 11, 101-105.

Koval,M., Pagano,R.E., 1991. Intracellular transport and metabolism of sphingomyelin. Biochim. Biophys. Acta 1082, 113-125.

Krebs,N.F., Hambidge,K.M., 2007. Complementary feeding: clinically relevant factors affecting timing and composition. Am J Clin Nutr. 85, 639S-645S.

Kronin,V., Winkel,K., Suss,G., Kelso,A., Heath,W., Kirberg,J., von Boehmer,H., Shortman,K., 1996a. A subclass of dendritic cells regulates the response of naive CD8 T cells by limiting their IL-2 production. J. Immunol. 157, 3819-3827.

Krug,A., French,A.R., Barchet,W., Fischer,J.A., Dzionek,A., Pingel,J.T., Orihuela,M.M., Akira,S., Yokoyama,W.M., Colonna,M., 2004. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. Immunity. 21, 107-119.

Kumar,H., Kawai,T., Akira,S., 2009. Pathogen recognition in the innate immune response. Biochem. J. 420, 1-16.

Labeur,M.S., Roters,B., Pers,B., Mehling,A., Luger,T.A., Schwarz,T., Grabbe,S., 1999. Generation of tumor immunity by bone marrow-derived dendritic cells correlates with dendritic cell maturation stage. J. Immunol. 162, 168-175.

Lane,P.J., Brocker,T., 1999. Developmental regulation of dendritic cell function. Curr. Opin. Immunol. 11, 308-313.

Lang,F., Gulbins,E., Lerche,H., Huber,S.M., Kempe,D.S., Foller,M., 2008a. Eryptosis, a window to systemic disease. Cell Physiol Biochem 22, 373-380.

Lang,K.S., Myssina,S., Brand,V., Sandu,C., Lang,P.A., Berchtold,S., Huber,S.M., Lang,F., Wieder,T., 2004. Involvement of ceramide in hyperosmotic shock-induced death of erythrocytes. Cell Death. Differ. 11, 231-243.

Lang,P.A., Huober,J., Bachmann,C., Kempe,D.S., Sobiesiak,M., Akel,A., Niemoeller,O.M., Dreischer,P., Eisele,K., Klarl,B.A., Gulbins,E., Lang,F., Wieder,T., 2006. Stimulation of erythrocyte phosphatidylserine exposure by paclitaxel. Cell Physiol Biochem. 18, 151-164.

Lang,P.A., Schenck,M., Nicolay,J.P., Becker,J.U., Kempe,D.S., Lupescu,A., Koka,S., Eisele,K., Klarl,B.A., Rubben,H., Schmid,K.W., Mann,K., Hildenbrand,S., Hefter,H., Huber,S.M., Wieder,T., Erhardt,A., Haussinger,D., Gulbins,E., Lang,F., 2007. Liver cell death and anemia in Wilson disease involve acid sphingomyelinase and ceramide. Nat. Med. 13, 164-170.

Larghero,P., Vene,R., Minghelli,S., Travaini,G., Morini,M., Ferrari,N., Pfeffer,U., Noonan,D.M., Albini,A., Benelli,R., 2007b. Biological assays and genomic analysis reveal lipoic acid modulation of endothelial cell behavior and gene expression. Carcinogenesis 28, 1008-1020.

Lauwerys,B.R., Garot,N., Renauld,J.C., Houssiau,F.A., 2000. Cytokine production and killer activity of NK/T-NK cells derived with IL-2, IL-15, or the combination of IL-12 and IL-18. J. Immunol. 165, 1847-1853.

Lee,S.Y., Jin,H.H., 2008b. Inhibitory activity of natural antimicrobial compounds alone or in combination with nisin against Enterobacter sakazakii. Lett Appl Microbiol 47, 315-321.

Lehner,T., 2008. Special regulatory T cell review: The resurgence of the concept of contrasuppression in immunoregulation. Immunology 123, 40-44.

Levade,T., Jaffrezou,J.P., 1999. Signalling sphingomyelinases: which, where, how and why? Biochim. Biophys. Acta 1438, 1-17.

Leverkus,M., Walczak,H., McLellan,A., Fries,H.W., Terbeck,G., Brocker,E.B., Kampgen,E., 2000. Maturation of dendritic cells leads to up-regulation of cellular FLICE-inhibitory protein and concomitant down-regulation of death ligand-mediated apoptosis. Blood 96, 2628-2631.

Li,M.O., Flavell,R.A., 2008a. Contextual regulation of inflammation: a duet by transforming growth factor-beta and interleukin-10. Immunity. 28, 468-476.

Linsley,P.S., Clark,E.A., Ledbetter,J.A., 1990. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. Proc. Natl. Acad. Sci. U. S. A 87, 5031-5035.

Litonjua,A.A., Rifas-Shiman,S.L., Ly,N.P., Tantisira,K.G., Rich-Edwards,J.W., Camargo,C.A., Jr., Weiss,S.T., Gillman,M.W., Gold,D.R., 2006. Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 y of age. Am J Clin Nutr. 84, 903-911.

Liu,Y.J., 2001b. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. Cell 106, 259-262.

Long,K.Z., Montoya,Y., Hertzmark,E., Santos,J.I., Rosado,J.L., 2006. A double-blind, randomized, clinical trial of the effect of vitamin A and zinc supplementation on diarrheal disease and respiratory tract infections in children in Mexico City, Mexico. Am J Clin Nutr. 83, 693-700.

Lundqvist,A., Nagata,T., Kiessling,R., Pisa,P., 2002b. Mature dendritic cells are protected from Fas/CD95-mediated apoptosis by upregulation of Bcl-X(L). Cancer Immunol. Immunother. 51, 139-144.

Lust,S., Vanhoecke,B., Janssens,A., Philippe,J., Bracke,M., Offner,F., 2005a. Xanthohumol kills B-chronic lymphocytic leukemia cells by an apoptotic mechanism. Mol Nutr. Food Res 49, 844-850.

Lutz,M.B., Schuler,G., 2002. Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? Trends Immunol. 23, 445-449.

Lyakh,L.A., Sanford,M., Chekol,S., Young,H.A., Roberts,A.B., 2005. TGF-beta and vitamin D3 utilize distinct pathways to suppress IL-12 production and modulate rapid differentiation of human monocytes into CD83+ dendritic cells. J Immunol 174, 2061-2070.

MacDonald,T.T., Monteleone,G., 2005. Immunity, inflammation, and allergy in the gut. Science 307, 1920-1925.

Macpherson,G., Kushnir,N., Wykes,M., 1999. Dendritic cells, B cells and the regulation of antibody synthesis. Immunol. Rev. 172, 325-334.

Mahmud,H., Mauro,D., Föller,M., Lang,F., 2009. Inhibitory effect of thymol on suicidal erythrocyte death. in preparation.

Maldonado-Lopez,R., De Smedt,T., Michel,P., Godfroid,J., Pajak,B., Heirman,C., Thielemans,K., Leo,O., Urbain,J., Moser,M., 1999. CD8alpha+ and CD8alpha- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. J. Exp. Med. 189, 587-592.

Maldonado-Lopez,R., Moser,M., 2001. Dendritic cell subsets and the regulation of Th1/Th2 responses. Semin. Immunol. 13, 275-282.

Marchetti,P., Castedo,M., Susin,S.A., Zamzami,N., Hirsch,T., Macho,A., Haeffner,A., Hirsch,F., Geuskens,M., Kroemer,G., 1996. Mitochondrial permeability transition is a central coordinating event of apoptosis. J. Exp. Med. 184, 1155-1160.

Marquez,C., Trigueros,C., Franco,J.M., Ramiro,A.R., Carrasco,Y.R., Lopez-Botet,M., Toribio,M.L., 1998. Identification of a common developmental pathway for thymic natural killer cells and dendritic cells. Blood 91, 2760-2771.

Marteau,P., Seksik,P., Lepage,P., Dore,J., 2004. Cellular and physiological effects of probiotics and prebiotics. Mini. Rev. Med. Chem. 4, 889-896.

Massadeh,A., Gharibeh,A., Omari,K., Al Momani,I., Alomari,A., Tumah,H., Hayajneh,W., 2009. Simultaneous Determination of Cd, Pb, Cu, Zn, and Se in Human Blood of Jordanian Smokers by ICP-OES. Biol Trace Elem. Res.

Matsue,H., Takashima,A., 1999. Apoptosis in dendritic cell biology. J. Dermatol. Sci. 20, 159-171.

Matsumoto,N., Riley,S., Fraser,D., Al Assaf,S., Ishimura,E., Wolever,T., Phillips,G.O., Phillips,A.O., 2006b. Butyrate modulates TGF-beta1 generation and function: potential renal benefit for Acacia(sen) SUPERGUM (gum arabic)? Kidney Int. 69, 257-265.

McLellan,A.D., Terbeck,G., Mengling,T., Starling,G.C., Kiener,P.A., Gold,R., Brocker,E.B., Leverkus,M., Kampgen,E., 2000. Differential susceptibility to CD95 (Apo-1/Fas) and MHC class II-induced apoptosis during murine dendritic cell development. Cell Death. Differ. 7, 933-938.

Medzhitov,R., Janeway,C.A., Jr., 1999. Innate immune induction of the adaptive immune response. Cold Spring Harb. Symp. Quant. Biol. 64, 429-435.

Mendes,V., Monteiro,R., Pestana,D., Teixeira,D., Calhau,C., Azevedo,I., 2008. Xanthohumol influences preadipocyte differentiation: implication of antiproliferative and apoptotic effects. J Agric. Food Chem. 56, 11631-11637.

Millard,A.L., Mertes,P.M., Ittelet,D., Villard,F., Jeannesson,P., Bernard,J., 2002. Butyrate affects differentiation, maturation and function of human monocyte-derived dendritic cells and macrophages. Clin Exp. Immunol 130, 245-255.

Mogensen,T.H., 2009. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin. Microbiol. Rev. 22, 240-73, Table.

Mohamad,N., Gutierrez,A., Nunez,M., Cocca,C., Martin,G., Cricco,G., Medina,V., Rivera,E., Bergoc,R., 2005. Mitochondrial apoptotic pathways. Biocell 29, 149-161.

Mohamed,A., Shoker,A., Bendjelloul,F., Mare,A., Alzrigh,M., Benghuzzi,H., Desin,T., 2003. Improvement of experimental allergic encephalomyelitis (EAE) by thymoquinone; an oxidative stress inhibitor. Biomed. Sci Instrum. 39, 440-445.

Monteghirfo,S., Tosetti,F., Ambrosini,C., Stigliani,S., Pozzi,S., Frassoni,F., Fassina,G., Soverini,S., Albini,A., Ferrari,N., 2008c. Antileukemia effects of xanthohumol in Bcr/Abl-transformed cells involve nuclear factor-kappaB and p53 modulation. Mol Cancer Ther. 7, 2692-2702.

Monteiro,R., Calhau,C., Silva,A.O., Pinheiro-Silva,S., Guerreiro,S., Gartner,F., Azevedo,I., Soares,R., 2008a. Xanthohumol inhibits inflammatory factor production and angiogenesis in breast cancer xenografts. J Cell Biochem 104, 1699-1707.

Monteiro,R., Faria,A., Azevedo,I., Calhau,C., 2007b. Modulation of breast cancer cell survival by aromatase inhibiting hop (Humulus lupulus L.) flavonoids. J Steroid Biochem Mol Biol 105, 124-130.

Murakami,M., Hirano,T., 2008b. Intracellular zinc homeostasis and zinc signaling. Cancer Sci. 99, 1515-1522.

Murphy,E., Imahashi,K., Steenbergen,C., 2005. Bcl-2 regulation of mitochondrial energetics. Trends Cardiovasc. Med. 15, 283-290.

Nagler-Anderson,C., 2001. Man the barrier! Strategic defences in the intestinal mucosa. Nat. Rev. Immunol. 1, 59-67.

Nakahara,T., Uchi,H., Urabe,K., Chen,Q., Furue,M., Moroi,Y., 2004a. Role of c-Jun N-terminal kinase on lipopolysaccharide induced maturation of human monocyte-derived dendritic cells. Int. Immunol. 16, 1701-1709.

Nasir,O., Artunc,F., Saeed,A., Kambal,M.A., Kalbacher,H., Sandulache,D., Boini,K.M., Jahovic,N., Lang,F., 2008a. Effects of gum arabic (Acacia senegal) on water and electrolyte balance in healthy mice. J Ren Nutr. 18, 230-238.

Nencioni,A., Garuti,A., Schwarzenberg,K., Cirmena,G., Dal Bello,G., Rocco,I., Barbieri,E., Brossart,P., Patrone,F., Ballestrero,A., 2006a. Proteasome inhibitor-induced apoptosis in human monocyte-derived dendritic cells. Eur. J. Immunol. 36, 681-689.

Newsholme,P., Keane,D., Welters,H.J., Morgan,N.G., 2007. Life and death decisions of the pancreatic beta-cell: the role of fatty acids. Clin. Sci. (Lond) 112, 27-42.

Nicolay,J.P., Schneider,J., Niemoeller,O.M., Artunc,F., Portero-Otin,M., Haik,G., Jr., Thornalley,P.J., Schleicher,E., Wieder,T., Lang,F., 2006. Stimulation of suicidal erythrocyte death by methylglyoxal. Cell Physiol Biochem. 18, 223-232.

Nicolo,C., Tomassini,B., Rippo,M.R., Testi,R., 2001. UVB-induced apoptosis of human dendritic cells: contribution by caspase-dependent and caspase-independent pathways. Blood 97, 1803-1808.

Niess,J.H., 2008. Role of mucosal dendritic cells in inflammatory bowel disease. World J. Gastroenterol. 14, 5138-5148.

Outten,C.E., O'Halloran,T.V., 2001. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. Science 292, 2488-2492.

Packham,G., Porter,C.W., Cleveland,J.L., 1996. c-Myc induces apoptosis and cell cycle progression by separable, yet overlapping, pathways. Oncogene 13, 461-469.

Pan,L., Becker,H., Gerhauser,C., 2005a. Xanthohumol induces apoptosis in cultured 40-16 human colon cancer cells by activation of the death receptor- and mitochondrial pathway. Mol Nutr. Food Res 49, 837-843.

Penna,G., Amuchastegui,S., Laverny,G., Adorini,L., 2007. Vitamin D receptor agonists in the treatment of autoimmune diseases: selective targeting of myeloid but not plasmacytoid dendritic cells. J Bone Miner. Res 22 Suppl 2, V69-V73.

Perrotta,C., De Palma,C., Clementi,E., 2008a. Nitric oxide and sphingolipids: mechanisms of interaction and role in cellular pathophysiology. Biol Chem. 389, 1391-1397.

Perrotta,C., De Palma,C., Clementi,E., 2008b. Nitric oxide and sphingolipids: mechanisms of interaction and role in cellular pathophysiology. Biol Chem. 389, 1391-1397.

Pettus,B.J., Chalfant,C.E., Hannun,Y.A., 2002. Ceramide in apoptosis: an overview and current perspectives. Biochim. Biophys. Acta 1585, 114-125.

Phillips,G.O., 1998. Acacia gum (Gum Arabic): a nutritional fibre; metabolism and calorific value. Food Addit. Contam 15, 251-264.

Pop,C., Salvesen,G.S., 2009. Human caspases: Activation, specificity and regulation. J. Biol. Chem.

Powell,J.D., 2006. The induction and maintenance of T cell anergy. Clin. Immunol. 120, 239-246.

Prasad,A.S., 1995. Zinc: an overview. Nutrition 11, 93-99.

Prasad,A.S., 2000a. Effects of zinc deficiency on Th1 and Th2 cytokine shifts. J Infect. Dis. 182 Suppl 1, S62-S68.

Prasad,A.S., Beck,F.W., Bao,B., Fitzgerald,J.T., Snell,D.C., Steinberg,J.D., Cardozo,L.J., 2007. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. Am J Clin. Nutr. 85, 837-844.

Quadri,S.M., Mahmud,H., Föller,M., Lang,F., 2009. Inhibition of suicidal erythrocyte death by xanthohumol. J Argic Food Chem -(submitted).

Randolph,G.J., Ochando,J., Partida-Sanchez,S., 2008. Migration of dendritic cell subsets and their precursors. Annu. Rev. Immunol. 26, 293-316.

Rasmussen,S.B., Reinert,L.S., Paludan,S.R., 2009. Innate recognition of intracellular pathogens: detection and activation of the first line of defense. APMIS 117, 323-337.

Razzaghi-Abyaneh,M., Shams-Ghahfarokhi,M., Yoshinari,T., Rezaee,M.B., Jaimand,K., Nagasawa,H., Sakuda,S., 2008a. Inhibitory effects of Satureja hortensis L. essential oil on growth and aflatoxin production by Aspergillus parasiticus. Int J Food Microbiol 123, 228-233.

Reis e Sousa, Stahl,P.D., Austyn,J.M., 1993a. Phagocytosis of antigens by Langerhans cells in vitro. J. Exp. Med. 178, 509-519.

Remondo,C., Cereda,V., Mostbock,S., Sabzevari,H., Franzusoff,A., Schlom,J., Tsang,K.Y., 2009. Human dendritic cell maturation and activation by a heat-killed recombinant yeast (Saccharomyces cerevisiae) vector encoding carcinoembryonic antigen. Vaccine 27, 987-994.

Reschner,A., Hubert,P., Delvenne,P., Boniver,J., Jacobs,N., 2008a. Innate lymphocyte and dendritic cell cross-talk: a key factor in the regulation of the immune response. Clin. Exp. Immunol. 152, 219-226.

Rescigno,M., Lopatin,U., Chieppa,M., 2008. Interactions among dendritic cells, macrophages, and epithelial cells in the gut: implications for immune tolerance. Curr. Opin. Immunol. 20, 669-675.

Rescigno,M., Martino,M., Sutherland,C.L., Gold,M.R., Ricciardi-Castagnoli,P., 1998. Dendritic cell survival and maturation are regulated by different signaling pathways. J. Exp. Med. 188, 2175-2180.

Rescigno,M., Rotta,G., Valzasina,B., Ricciardi-Castagnoli,P., 2001. Dendritic cells shuttle microbes across gut epithelial monolayers. Immunobiology 204, 572-581.

Reuter,S., Eifes,S., Dicato,M., Aggarwal,B.B., Diederich,M., 2008. Modulation of anti-apoptotic and survival pathways by curcumin as a strategy to induce apoptosis in cancer cells. Biochem. Pharmacol. 76, 1340-1351.

Richard,S.A., Zavaleta,N., Caulfield,L.E., Black,R.E., Witzig,R.S., Shankar,A.H., 2006c. Zinc and iron supplementation and malaria, diarrhea, and respiratory infections in children in the Peruvian Amazon. Am J Trop. Med Hyg. 75, 126-132.

Roepke,M., Diestel,A., Bajbouj,K., Walluscheck,D., Schonfeld,P., Roessner,A., Schneider-Stock,R., Gali-Muhtasib,H., 2007b. Lack of p53 augments thymoquinone-induced apoptosis and caspase activation in human osteosarcoma cells. Cancer Biol Ther. 6, 160-169.

Rooney,S., Ryan,M.F., 2005a. Effects of alpha-hederin and thymoquinone, constituents of Nigella sativa, on human cancer cell lines. Anticancer Res 25, 2199-2204.

Rooney,S., Ryan,M.F., 2005d. Modes of action of alpha-hederin and thymoquinone, active constituents of Nigella sativa, against HEp-2 cancer cells. Anticancer Res 25, 4255-4259.

Saemann,M.D., Parolini,O., Bohmig,G.A., Kelemen,P., Krieger,P.M., Neumuller,J., Knarr,K., Kammlander,W., Horl,W.H., Diakos,C., Stuhlmeier,K., Zlabinger,G.J., 2002. Bacterial metabolite interference with maturation of human monocyte-derived dendritic cells. J Leukoc. Biol 71, 238-246.

Salem,M.L., 2005a. Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. Int Immunopharmacol. 5, 1749-1770.

Sallusto,F., Cella,M., Danieli,C., Lanzavecchia,A., 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J. Exp. Med. 182, 389-400.

Sallusto,F., Lanzavecchia,A., 1999. Mobilizing dendritic cells for tolerance, priming, and chronic inflammation. J. Exp. Med. 189, 611-614.

Sallusto,F., Nicolo,C., De Maria,R., Corinti,S., Testi,R., 1996. Ceramide inhibits antigen uptake and presentation by dendritic cells. J. Exp. Med. 184, 2411-2416.

Sallusto,F., Palermo,B., Lenig,D., Miettinen,M., Matikainen,S., Julkunen,I., Forster,R., Burgstahler,R., Lipp,M., Lanzavecchia,A., 1999. Distinct patterns and kinetics of chemokine production regulate dendritic cell function. Eur. J. Immunol. 29, 1617-1625.

Schutze,S., Potthoff,K., Machleidt,T., Berkovic,D., Wiegmann,K., Kronke,M., 1992. TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown. Cell 71, 765-776.

Schuurhuis,D.H., Fu,N., Ossendorp,F., Melief,C.J., 2006. Ins and outs of dendritic cells. Int Arch Allergy Immunol 140, 53-72.

Schwartz,L.B., 2002. Mast cells and basophils. Clin. Allergy Immunol. 16, 3-42.

Scott,P., 1993. IL-12: initiation cytokine for cell-mediated immunity. Science 260, 496-497.

Sedlak,T.W., Oltvai,Z.N., Yang,E., Wang,K., Boise,L.H., Thompson,C.B., Korsmeyer,S.J., 1995. Multiple Bcl-2 family members demonstrate selective dimerizations with Bax. Proc. Natl. Acad. Sci. U. S. A 92, 7834-7838.

Seifarth,C.C., Hinkmann,C., Hahn,E.G., Lohmann,T., Harsch,I.A., 2008. Reduced frequency of peripheral dendritic cells in type 2 diabetes. Exp. Clin. Endocrinol. Diabetes 116, 162-166.

Sethi,G., Ahn,K.S., Aggarwal,B.B., 2008b. Targeting nuclear factor-kappa B activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. Mol Cancer Res 6, 1059-1070.

Sheng,X.Y., Hambidge,K.M., Zhu,X.X., Ni,J.X., Bailey,K.B., Gibson,R.S., Krebs,N.F., 2006. Major variables of zinc homeostasis in Chinese toddlers. Am J Clin Nutr. 84, 389-394.

Shevach,E.M., McHugh,R.S., Piccirillo,C.A., Thornton,A.M., 2001. Control of T-cell activation by CD4+ CD25+ suppressor T cells. Immunol. Rev. 182, 58-67.

Shoieb,A.M., Elgayyar,M., Dudrick,P.S., Bell,J.L., Tithof,P.K., 2003b. In vitro inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. Int J Oncol. 22, 107-113.

Shortman,K., Naik,S.H., 2007a. Steady-state and inflammatory dendritic-cell development. Nat. Rev. Immunol. 7, 19-30.

Silva,M.A., 2009. Intestinal dendritic cells and epithelial barrier dysfunction in Crohn's disease. Inflamm. Bowel. Dis. 15, 436-453.

Smith,C.M., Wilson,N.S., Waithman,J., Villadangos,J.A., Carbone,F.R., Heath,W.R., Belz,G.T., 2004. Cognate CD4(+) T cell licensing of dendritic cells in CD8(+) T cell immunity. Nat. Immunol. 5, 1143-1148.

Smith,E.L., Schuchman,E.H., 2008a. The unexpected role of acid sphingomyelinase in cell death and the pathophysiology of common diseases. FASEB J 22, 3419-3431.

Smith,E.L., Schuchman,E.H., 2008b. The unexpected role of acid sphingomyelinase in cell death and the pathophysiology of common diseases. FASEB J 22, 3419-3431.

Sopjani,M., Foller,M., Dreischer,P., Lang,F., 2008a. Stimulation of eryptosis by cadmium ions. Cell Physiol Biochem 22, 245-252.

Sopjani,M., Foller,M., Gulbins,E., Lang,F., 2008b. Suicidal death of erythrocytes due to selenium-compounds. Cell Physiol Biochem 22, 387-394.

Stammati,A., Bonsi,P., Zucco,F., Moezelaar,R., Alakomi,H.L., von Wright,A., 1999. Toxicity of selected plant volatiles in microbial and mammalian short-term assays. Food Chem. Toxicol 37, 813-823.

Steinbrink,K., Wolfl,M., Jonuleit,H., Knop,J., Enk,A.H., 1997. Induction of tolerance by IL-10-treated dendritic cells. J. Immunol. 159, 4772-4780.

Steinman,R.M., 1991a. The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. 9, 271-296.

Steinman,R.M., 2007. Dendritic cells: understanding immunogenicity. Eur. J. Immunol. 37 Suppl 1, S53-S60.

Steinman,R.M., Nussenzweig,M.C., 2002a. Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. Proc. Natl. Acad. Sci. U. S. A 99, 351-358.

Steinman,R.M., Pack,M., Inaba,K., 1997. Dendritic cells in the T-cell areas of lymphoid organs. Immunol. Rev. 156, 25-37.

Suzuki,Y., Nakamura,S., Sugiyama,K., Furuta,H., 1987. Differences of superoxide production in blood leukocytes stimulated with thymol between human and non-human primates. Life Sci 41, 1659-1664.

Szegezdi,E., Macdonald,D.C., Ni,C.T., Gupta,S., Samali,A., 2009. Bcl-2 family on guard at the ER. Am. J. Physiol Cell Physiol 296, C941-C953.

Szentandrassy,N., Szigeti,G., Szegedi,C., Sarkozi,S., Magyar,J., Banyasz,T., Csernoch,L., Kovacs,L., Nanasi,P.P., Jona,I., 2004. Effect of thymol on calcium handling in mammalian ventricular myocardium. Life Sci 74, 909-921.

Tabas,I., 1999a. Secretory sphingomyelinase. Chem. Phys. Lipids 102, 123-130.

Takahashi,T., Chiba,S., Nieda,M., Azuma,T., Ishihara,S., Shibata,Y., Juji,T., Hirai,H., 2002. Cutting edge: analysis of human V alpha 24+CD8+ NK T cells activated by alpha-galactosylceramide-pulsed monocyte-derived dendritic cells. J. Immunol. 168, 3140-3144.

Takeda,K., Akira,S., 2005b. Toll-like receptors in innate immunity. Int. Immunol. 17, 1-14.

Tarbell,K.V., Yamazaki,S., Steinman,R.M., 2006. The interactions of dendritic cells with antigen-specific, regulatory T cells that suppress autoimmunity. Semin. Immunol 18, 93-102.

Taylor,A., Verhagen,J., Blaser,K., Akdis,M., Akdis,C.A., 2006. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells. Immunology 117, 433-442.

Tekeoglu,I., Dogan,A., Ediz,L., Budancamanak,M., Demirel,A., 2007. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. Phytother. Res 21, 895-897.

Telford,W.G., Fraker,P.J., 1995. Preferential induction of apoptosis in mouse CD4+CD8+ alpha beta TCRloCD3 epsilon lo thymocytes by zinc. J. Cell Physiol 164, 259-270.

Tiss,A., Carriere,F., Verger,R., 2001. Effects of gum arabic on lipase interfacial binding and activity. Anal. Biochem. 294, 36-43.

Trivedi,S.G., Lloyd,C.M., 2007. Eosinophils in the pathogenesis of allergic airways disease. Cell Mol. Life Sci. 64, 1269-1289.

Trombetta,E.S., Mellman,I., 2005. Cell biology of antigen processing in vitro and in vivo. Annu. Rev. Immunol. 23, 975-1028.

Undeger,U., Basaran,A., Degen,G.H., Basaran,N., 2009a. Antioxidant activities of major thyme ingredients and lack of (oxidative) DNA damage in V79 Chinese hamster lung fibroblast cells at low levels of carvacrol and thymol. Food Chem. Toxicol.

VALLEE,B.L., GIBSON,J.G., 1948. The zinc content of normal human whole blood, plasma, leucocytes, and erythrocytes. J Biol Chem. 176, 445-457.

van Duivenvoorde,L.M., Han,W.G., Bakker,A.M., Louis-Plence,P., Charbonnier,L.M., Apparailly,F., van der Voort,E.I., Jorgensen,C., Huizinga,T.W., Toes,R.E., 2007. Immunomodulatory dendritic cells inhibit Th1 responses and arthritis via different mechanisms. J Immunol 179, 1506-1515.

van Etten,E., Mathieu,C., 2005. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. J Steroid Biochem Mol Biol 97, 93-101.

Vanhoecke,B., Derycke,L., Van,M., V, Depypere,H., De Keukeleire,D., Bracke,M., 2005a. Antiinvasive effect of xanthohumol, a prenylated chalcone present in hops (Humulus lupulus L.) and beer. Int J Cancer 117, 889-895.

Vassiliou,E., Sharma,V., Jing,H., Sheibanie,F., Ganea,D., 2004a. Prostaglandin E2 promotes the survival of bone marrow-derived dendritic cells. J. Immunol. 173, 6955-6964.

Vivier,E., 2006. What is natural in natural killer cells? Immunol. Lett. 107, 1-7.

Vogel,S., Heilmann,J., 2008. Synthesis, cytotoxicity, and antioxidative activity of minor prenylated chalcones from Humulus lupulus. J Nat. Prod. 71, 1237-1241.

von Issendorff,B., Cheshnovsky,O., 2005. Metal to insulator transitions in clusters. Annu. Rev. Phys. Chem. 56, 549-580.

Wallet,M.A., Sen,P., Tisch,R., 2005. Immunoregulation of dendritic cells. Clin. Med. Res. 3, 166-175.

Wang,B., Morinobu,A., Horiuchi,M., Liu,J., Kumagai,S., 2008a. Butyrate inhibits functional differentiation of human monocyte-derived dendritic cells. Cell Immunol 253, 54-58.

Wang,J., Zheng,L., Lobito,A., Chan,F.K., Dale,J., Sneller,M., Yao,X., Puck,J.M., Straus,S.E., Lenardo,M.J., 1999. Inherited human Caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. Cell 98, 47-58.

Wang,K., Mahmud,H., Foller,M., Biswas,R., Lang,K.S., Bohn,E., Gotz,F., Lang,F., 2008b. Lipopeptides in the triggering of erythrocyte cell membrane scrambling. Cell Physiol Biochem 22, 381-386.

Wang,Q., Ding,Z.H., Liu,J.K., Zheng,Y.T., 2004. Xanthohumol, a novel anti-HIV-1 agent purified from Hops Humulus lupulus. Antiviral Res 64, 189-194.

Weiss,J.H., Sensi,S.L., 2000. Ca2+-Zn2+ permeable AMPA or kainate receptors: possible key factors in selective neurodegeneration. Trends Neurosci. 23, 365-371.

Weiss,J.H., Sensi,S.L., Koh,J.Y., 2000. Zn(2+): a novel ionic mediator of neural injury in brain disease. Trends Pharmacol. Sci. 21, 395-401.

Willems,F., Amraoui,Z., Vanderheyde,N., Verhasselt,V., Aksoy,E., Scaffidi,C., Peter,M.E., Krammer,P.H., Goldman,M., 2000. Expression of c-FLIP(L) and resistance to CD95-mediated apoptosis of monocyte-derived dendritic cells: inhibition by bisindolylmaleimide. Blood 95, 3478-3482.

Williams,M.E., Chang,T.L., Burke,S.K., Lichtman,A.H., Abbas,A.K., 1991. Activation of functionally distinct subsets of CD4+ T lymphocytes. Res. Immunol. 142, 23-28.

Xia,C.Q., Peng,R., Beato,F., Clare-Salzler,M.J., 2005. Dexamethasone induces IL-10-producing monocyte-derived dendritic cells with durable immaturity. Scand. J. Immunol. 62, 45-54.

Yang,A.S., Lattime,E.C., 2003b. Tumor-induced interleukin 10 suppresses the ability of splenic dendritic cells to stimulate CD4 and CD8 T-cell responses. Cancer Res. 63, 2150-2157.

Yang,E., Zha,J., Jockel,J., Boise,L.H., Thompson,C.B., Korsmeyer,S.J., 1995. Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. Cell 80, 285-291.

Yang,J.Y., Della-Fera,M.A., Rayalam,S., Baile,C.A., 2007. Effect of xanthohumol and isoxanthohumol on 3T3-L1 cell apoptosis and adipogenesis. Apoptosis. 12, 1953-1963.

Yang,J.Y., Della-Fera,M.A., Rayalam,S., Baile,C.A., 2008. Enhanced effects of xanthohumol plus honokiol on apoptosis in 3T3-L1 adipocytes. Obesity. (Silver. Spring) 16, 1232-1238.

Yi,T., Cho,S.G., Yi,Z., Pang,X., Rodriguez,M., Wang,Y., Sethi,G., Aggarwal,B.B., Liu,M., 2008. Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. Mol Cancer Ther. 7, 1789-1796.

Younes,H., Garleb,K., Behr,S., Remesy,C., Demigne,C., 1995. Fermentable fibers or oligosaccharides reduce urinary nitrogen excretion by increasing urea disposal in the rat cecum. J. Nutr. 125, 1010-1016.

Zeitz,M., Schieferdecker,H.L., James,S.P., Riecken,E.O., 1990. Special functional features of T-lymphocyte subpopulations in the effector compartment of the intestinal mucosa and their relation to mucosal transformation. Digestion 46 Suppl 2, 280-289.

Zha,J., Harada,H., Yang,E., Jockel,J., Korsmeyer,S.J., 1996. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). Cell 87, 619-628.

Zhao,F., Nozawa,H., Daikonnya,A., Kondo,K., Kitanaka,S., 2003. Inhibitors of nitric oxide production from hops (Humulus lupulus L.). Biol. Pharm. Bull. 26, 61-65.

CURICULUM VITAE

Basic information

Family name:	Nguyen Thi
Given names:	XUAN
Date of birth:	20-12-1976
Place of birth:	Thai Thuy - Thai Binh- VIETNAM
Nationality:	Vietnamese
Sex:	Female
Marital status:	Married
Language:	- *Mother tongue:* Vietnamese
	- *Foreign languages:* English, German
Address:	P19- TT 198A - Lang Thuong - Dong Da - Hanoi - Vietnam
Current place of work:	Postdoctoral Researcher, Physiology Institute, Gmelinstrasse 5, 72076, Tuebingen, Germany
	Phone: +49 7071 29 77359
	Fax: +49 7071 29 5618
	E-mail: xthlinh76@yahoo.com

Education

- **1991 - 1994**

- *Institution:* Thai Binh secondary school *(Gymnasium)*
- *Diploma:* High-school finals *(Abitur) (Very good)*

- **1994 - 1998:**

- *Institution:* Hanoi National University, Hanoi University for Teacher training
- *Title of thesis:* "Researching the Barm for industrial rearing"
- *Diploma:* Bachelor of Science (BSc) *(Very good)*

- **1998 - 2001:**

- *Institution:* Hanoi University of Foreign Languages, Vietnam.
- *Diploma:* Bachelor of English *(Fairly good)*

- **2002 - 2003:**

- *Institution:* Le Ngoc Han Foreign Languages Centre, Hanoi, Vietnam.
- *Diploma:* Certificate on Deutsch Grünstufe I-II

- **2003 - 2006:**

- *Institution:* Faculty of Biology, University of Tuebingen, Germany.
- *Diploma:* Diploma of Biology

- **September 2006 to 2nd November 2009:**

- *Institution:* Department of physiology, University of Tuebingen, Germany.
- *Diploma:* Doctor of natural sciences (Dr. re. Nat) (Very good)
- *Title of thesis:* "Regulation of Dendritic cells by Nutrients"

Career

- **1998 -2003**

  Research assistant at the center of microbiology under the Hanoi National University

- **2003-2006**

  Practice and research assistant at the Tropical Medicine Institute, University of Tuebingen

- **September 2006 to 2nd November 2009**

  PhD student at the department of physiology, University of Tuebingen

- **November 2009 to present**

  Postdoctoral Researcher, Laboratory of Immune Cell Culture, Institute of Physiology I, University of Tuebingen, Tuebingen, Germany.

Working experiences

- proficient in software for word processing, statistical and gene-technological applications: STATVIEW, GENE RUNNER, ORIGIN, BioEdit, Adobe Photoshop, CorelDRAW, CANVAS, Quantity One, Office etc.
- knowledge of physiology, molecular biology, microbiology, genetics, immunology, Hepatitis B Virus (HBV), etc.
- familiar with luciferase measurement, DNA sequencing, etc.
- experienced on isolating DNA, RNA from whole blood and cells and tissue; genotyping, enzyme-linked immunosorbent assay (ELISA); polymerase chain reaction (PCR), Reverse Transcription-PCR, Real time- PCR; FACS for Dendritic cells, Mast cells, T cells, B cells; Confocal; Culture of bone marrow derived-dendritic cells and mast cells, Western Blotting; siRNA; isolating Dendritic Cells and T cells from spleen (MACS) etc.
- Highly self-motivated, high inter-personal skills and leadership capability.

PUBLICATIONs

1. **Xuan,N.T**.; Shumilina,E.; Gu,S.; Götz,F.; Lang,F. Triggering of dendritic cell apoptosis by xanthohumol. Molecular Nutrition and Food Research- ***In press.***

2. **Xuan,N.T**.; Song,L.H.; Toan,N.L.; Binh,V.Q.; Boldt,A.B.; Kremsner,P.G.; Kun,J.F.J. "Association of two variants of the interferon-alpha receptor-1 gene with the presentation of hepatitis B virus infection." European Cytokine Network 19.4 (2008): 204-10.

3. **Xuan,N.T**.; Shumilina,E.; Matzner,N.; Zemtsova,I.M.; Bidermann,T.; Götz,F; Lang,F. Ca^{2+} dependent functions in peptidoglycan-stimulated mouse dendritic cells. Cellular Physiology and Biochemistry 16.6 (2009).

4. **Xuan,N.T**.; Shumilina,E.; Qadri,S.M; Götz,F.; Lang,F. Effects of thymoquinone on mouse dendritic cells. Cellular Physiology and Biochemistry. ***In press.***

5. Matzner,N.; Zemtsova,I.M.; **Nguyen,T.X**.; Duszenko,M.; Shumilina,E.; Lang,F."Ion Channels Modulating Mouse Dendritic Cell Functions." Journal of Immunology 181.10 (2008): 6803-09.

6. Shumilina,E.; Zahir,N.; **Xuan,N.T**.; Lang,F."Phosphoinositide 3-kinase dependent regulation of Kv channels in dendritic cells." Cellular Physiology and Biochemistry 20.6 (2007): 801-08.

7. Song,L.H.; Toan,N.L.; **Xuan,N.T**.; Uhlemann,A.C.; Boldt,A.B.W.; Duy,D.N.; Binh,V.Q.; Kremsner,P.G.; Kun,J.F.J."A promoter polymorphism in the interferon alpha-2 gene is associated with the clinical presentation of hepatitis B." Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis 601.1-2 (2006): 137-43.

8. **Xuan,N.T**.; Shumilina,E.; Kempe,D.S.; Gulbins,E.; Lang,F. Sphingomyelinase dependent apoptosis of dendritic cells following treatment with amyloid peptides. (Journal of Neuroimmunology- ***Resubmitted.***

9. **Xuan,N.T**.; Shumilina,E.; Götz,F.; Lang,F. Role of acidic sphingomyelinase in thymol-mediated dendritic cell death. (Journal of Inflamation- ***under revision).***

10. **Xuan,N.T**.; Shumilina,E.; Nasir,O; Bobbala,D.; Götz,F.; Lang,F. Stimulation of mouse dendritic cells by gum arabic. Cellular Physiology and Biochemistry- ***under revision).***

11. **Xuan,N.T**.; Zahir,N.; Kuhl,D.; Lang,F.; Shumilina,E. Serum- and Glucocorticoid-inducible kinase1 SGK1 in the regulation of dendritic cell functions. ***In preparation.***

12. **Xuan,N.T**.; Shumilina,E.; Kempe, DS.; Matzner,N.; Lang,F. Klotho dependent regulation of Ca^{2+} entry into and maturation of dendritic cells. ***In preparation.***

13. Shumilina,E.; **Xuan,N.T**.; Matzner,N.; Bhandaru,M.; Zemtsova,I.M.; Lang,F. Regulation of calcium signaling in dendritic cells by 1,25-Dihydroxyvitamin D3. FASEB-- ***under revision).***

14. Shumilina,E.; **Xuan,N.T**.; Gu,S.; Götz,F.; Lang,F. Zinc induced suicidal death of mouse dendritic cells. (Apoptosis- ***under revision).***

15. Matzner,N.; Shumilina,E.; **Xuan,N.T**.; Bhandaru,M.; Lang,F. Effect of dexamethasone on calcium signaling in dendritic cells. (Journal of Immunology- ***Submitted).***

Tuebingen, 9th November 2009