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Conference review

12th international TNF conference: The good, the bad and the scientists

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ABSTRACT

Members of the TNF superfamily control numerous aspects of immune defense as well as various processes of homeostasis and embryonic development. Recent advances in our knowledge of both the beneficial and the deleterious activities of these cytokines were thoroughly discussed at this conference. Participants presented new information about signaling mechanisms that these cytokines activate, with special attention to cell-death regulation, ubiquitination of signaling-proteins as a means of regulating their function, and complex systems of gene and signaling regulation. Sessions were devoted specifically to aberrations in functions of the TNF-family that contribute to the pathology of infectious, autoimmune and neurodegenerative diseases and to cancer, and to the application of our knowledge to therapy.

Contents

1. Introduction

1.1. General introduction

The surge in research activities that was prompted in 1985 by the cloning of tumor necrosis factor (TNF) continues to grow. The range of known activities of TNF, of the large families of related cytokines (the TNF superfamily), and of receptors to which they bind (the TNF/NGF receptor family) keeps expanding, and so does the community of scientists who study them. Communication within this large international community is greatly assisted by its biennial international conferences. The present conference, the 12th in the series, was held in San Lorenzo de El Escorial near Madrid, Spain, in April 2009. As highlighted by the logo adopted for this meeting—paintings of scenes from hell and heaven (from the "Haywain" Triptych of Hieronymus Bosch that hangs in the El Escorial monastery)—the major challenge in our research field from the start has been to find ways of controlling the diametrically opposing consequences of the TNF-family functions, such as killing cells versus inducing cell resistance to death,

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triggering inflammation versus arresting it, enhancing angiogenesis versus inducing vascular damage, and destroying tumors versus promoting their growth (Fig. 1). Although these puzzling antitheses are likely to demand our close attention for many years to come, there was a definite feeling at this meeting that the vast amount of knowledge already gained has set us on the path towards deeper understanding. It seems that we may have reached the stage at which combining the acquired information with new

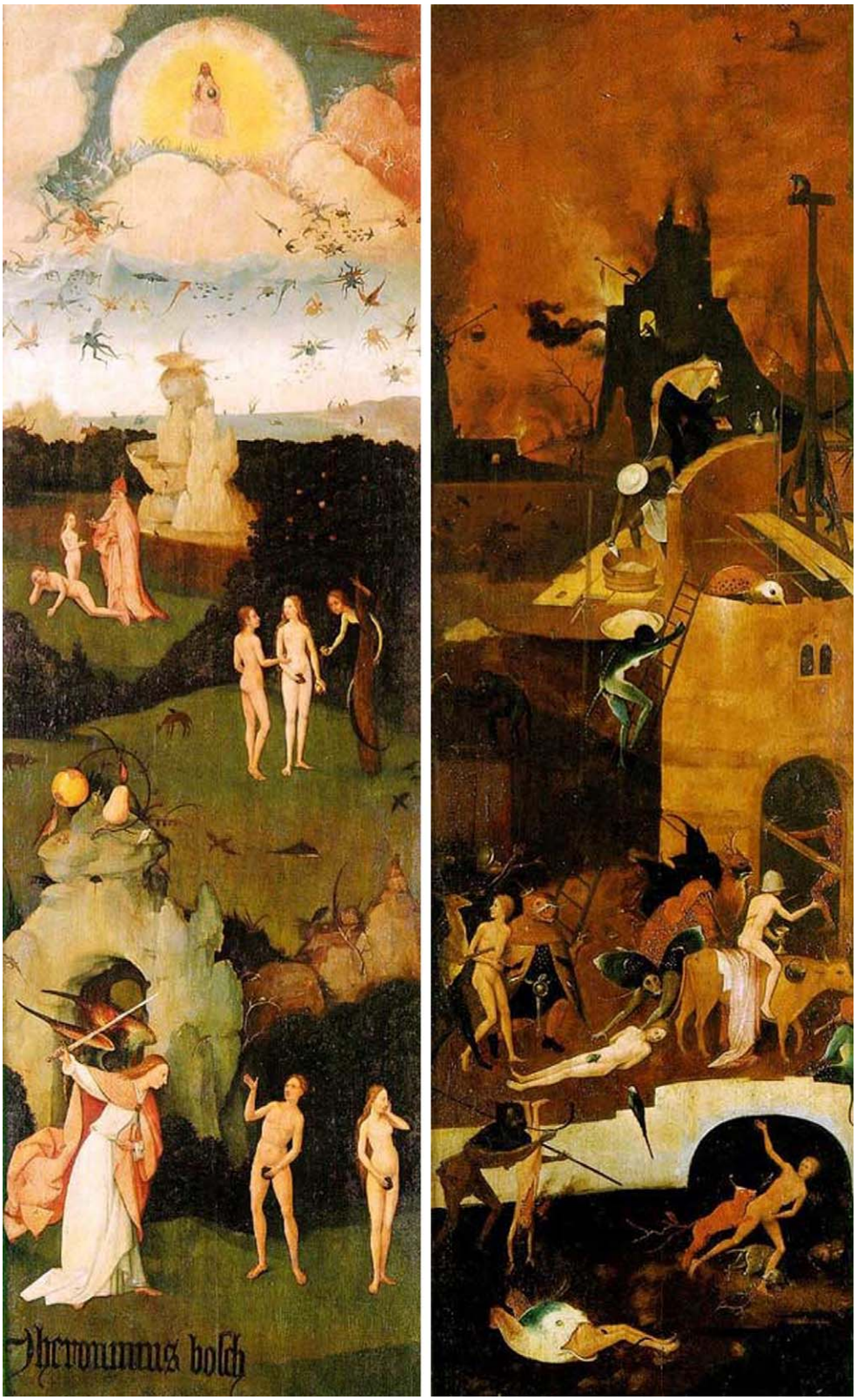

Fig. 1. The logo chosen for the 12th international TNF conference— scenes from heaven and hell from the "Haywain" Triptych of Hieronymus Bosch in the El Escorial monastery— highlights the diametrically opposing functions that can be mediated by individual members of the TNF-family, with corresponding deleterious or beneficial consequences, such as the killing of cells versus the induction of cell resistance to death, and the triggering of inflammation versus its arrest.

ways of thinking might make it possible to define, at least in part, the basis for these dualities, and to harness this knowledge for the design of effective therapies.

1.2. Complex patterns of gene induction and their control via microRNAs

"Where is the wisdom we have lost in knowledge?"[1] In the initial phase of study of the TNF-family there was the feeling that as the amount of knowledge about this family increases, our understanding of its significance decreases. The original, easily grasped notion of TNF as a cell-killing or anti-tumor molecule was replaced by a complexity that appeared at first to make little physiological sense and whose analysis in causal deterministic terms seemed beyond our ability. A repeating theme at this conference was that relatively novel approaches, for example genome-wide expression profiling, yielding comprehensive views of entire sets of induced molecular changes rather than only one, might allow us to make sense of this complexity. This notion was introduced in the keynote lecture by David Baltimore. Addressing the multiplicity and heterogeneity of changes in gene activation in cells treated by TNF, he presented findings on groups of genes with distinct kinetics of up- and down-regulation corresponding to different phases of the inflammatory response and showed that the waves of gene expression during inflammation are to large extent determined by variations in the rates of mRNA degradation rather than of synthesis. An important determinant controlling these variations is the induction of specific microRNAs (miRNAs). David Baltimore discussed the functions of miR-146a and miR-155, micro-RNAs induced by LPS and other inflammatory signals, which not only restrict inflammation but also regulate cell growth. miR-146a is a tumor suppressor that regulates cell growth, whereas miR-155 acts as oncogene. The multiple functions of miR-146a were further discussed by Mark Boldin. Lessons from other fields were presented by Yosef Yarden who, in discussing the contribution to cancer made by epidermal growth factor (EGF) receptor signaling, described the interactions of functionally different inducible genes affecting the cellular response to these receptors, and by Luke O'Neill, who showed how miR-21 helps to restrict inflammation induced by Toll-like receptors (TLRs). miRNAs also contribute to regulation of both the TNF-mediated cell-death induction (Juan Patrón) and expression of adhesion proteins by endothelial cells (Yajaira Suárez). An important question discussed was whether miRNAs achieve their physiological impact by tuning only a few cardinal genes, or whether every one of their targets (sometimes hundreds) is instrumental in generating the intended output.

The numerous feedback mechanisms affecting the signals induced by the TNF-family generate an intricate "signaling memory". The nature, duration, and dynamics of the signaling in response to each ligand are variably affected by prior exposure to that ligand and by cross-talk with signals generated by other cytokines. Our current ability to describe, in a comprehensive and formalized manner, the complex patterns of activation of different signaling molecules and of gene regulation by the TNF-family was the subject of a round-table discussion chaired by Mark Boldin, Alexander Hoffmann and Leroy Hood. The focus was on elucidating the rules by which the outcome of these patterns is determined with respect to important cellular decisions (for example, whether or not the cellular response would result in death, or which particular subset of NF-κB-regulated genes would be activated). Testing of mathematical models against experimental data was described for the NF-κB signaling system (Alexander Hoffmann), TNF-induced pathways (Tomoko Asaoka), and Fas-associated DISC (Inna Lavrik), as well as for the interplay between TNF- and Fas ligand (FasL)-induced signaling that results in sensitization by TNF to FasL-induced apoptosis (Kathrin Schmich).

2. Cellular and physiological functions of the TNF superfamily

2.1. Role of the TNF-family in tissue modeling and remodeling in health and disease

Among the huge range of activities mediated by TNF-family members, their ability to induce destruction as well as construction or reconstruction of tissues remains their most unique function. It is also (as reflected in the name of the family's founding member—"tumor necrosis factor"—which alludes to that kind of activity) the most astounding one. The ability of several members to cause both breakdown and buildup of tissues, while also initiating various functional changes unrelated to this remodeling role, illustrates the immensity of the challenge to our proficiency as scientists to analyze and understand the complex mechanistic processes underlying the family's multiple functions.

The first session at this meeting, chaired by Nuncy Ruddle and Joseph Penninger, was devoted to the functions of the TNF-family in tissue modeling. The functions of RANK ligand, its cell-bound receptor RANK, and its inhibitory soluble receptor OPG as key regulators of tissue modeling and remodeling were described by Penninger. RANK regulates bone dissolution through osteoclast activation, and is also required for the induction of embryonic lymph node development as well as of thymic microenvironment and lactating mammary gland development during pregnancy. A recent surprising addendum to the activities of the RANK ligand is its role as an essential intermediary in fever induction by inflammatory mediators such as TNF or IL6. Henry Mueller reported that RANK also regulates hair follicle development by inducing nuclear translocation of Bcl3, with consequent activation of NF-κB. Marja Mikkola presented evidence that a crucial role in mediating the embryonic development of hair is played by Troy (a member of the TNF/NGF receptor family), similar to the better-known role of the receptor Edar but at a different developmental phase. She also described two major molecular targets of Edar (dkk4 and lrp4), which are apparently located downstream of NF-κB activation, yet are dependent, in addition, on co-activation of the Wnt pathway. Interestingly, both of these target proteins seem to inhibit induction of placodes of ectodermal organs and thus enable Edar to restrict its own stimulatory effect on placode induction.

A similar duality of antagonizing effects characterizes many of the functions of TNF, underlying its role as inducer of tissue remodeling in inflammatory processes. Christopher Hughes reported that the ability of TNF to induce hemorrhagic necrosis (by triggering capillary destruction) and then regeneration of the capillary bed occurs through opposing responses of endothelial cells to TNF, defined by the duration of their exposure to it: continuous exposure induces changes such as arrest of VEGF receptor-2 signaling, resulting in arrest of angiogenesis, whereas intermittent exposure triggers sprouting of tip cells through NF-κB-mediated induction of the Notch ligand, jagged-1, as well as of PDGFB and VEGF receptor-2.

TWEAK and its non-death domain-containing receptor Fn14 somewhat resemble TNF and TNFR1 in their pleiotropicity of function; however, because the expression of Fn14 depends on inducing stimuli (while TNFR1 expression is ubiquitous and largely constitutive), its activity is more restricted spatially. This ligand–receptor couple, which occurs in species ranging from Zebra fish to humans and exhibits high structural conservation of the binding interface in Fn14 (Maria Pellegrini), has important functions in both physiological and pathological tissue remodeling. Linda

[1] From *The Rock*, T. S. Eliot.

Burkly, Ana Sanz, Gerald Atkins and Martin Ehrenschwender discussed its activities in promoting pathological damage of intestinal epithelium in injury models, bone remodeling in inflammation (synergistically with TNF, whose own cell-death-inducing function is enhanced by TWEAK-induced TRAF2 sequestration), tissue regeneration of the partially resected liver and the gamma-irradiated intestinal epithelium, and renal tubular cell proliferation (and thereby size readjustment) in the remaining kidney after surgical removal of the other.

Nancy Ruddle discussed the role of TNF-family members, particularly LTα and LTβ, in controlling lymphoid organ generation during embryogenesis and at sites of inflammation in the adult organism, as well as the involvement of induced vascularization in these processes.

2.2. Pirating of homeostatic and proinflammatory roles of TNF-family members as causes of tumor promotion

The destructive/constructive duality of TNF activity in inflammation is recapitulated in cancer, as reflected in its ability both to destroy tumors and to promote their development. A session co-chaired by Frances Balkwill and Avi Ashkenazi centered on these two opposing functions, which are also mediated by other TNF-family members. Most of the presentations about the anti-tumor functions of the family dealt with attempts at translation (see Section 4.2 below), while presentations about their tumor-promoting activities were focused on new findings about underlying mechanisms. With regard to the latter activities, the general message was that since these reflect "hijacking" of the family's remarkably wide range of functions related to immune defense and homeostasis, the tumor-promoting role of the TNF-family also involves wide heterogeneity of mechanisms. Besides TNF itself, family members that contribute in various ways to tumor promotion and metastasis include APRIL (in mammary cancer, discussed by Araceli Garcia-Castro), RANK ligand (in mammary and prostate cancers, reported by Bill Dougall), and the LTβR ligands, LTαβ and LIGHT (in hepatocellular carcinoma, reported by Mathias Heikenwalder). The presented mechanisms included direct impact of ligands on the tumor cells themselves, as in TNF effects on ovarian cancer cells (Balkwill) and hepatocellular carcinoma induction by LTβR triggering (Heikenwalder), or on tumor-associated immune cells as in induction by TNF of myeloid-derived suppressor cells (Zhihai Qin). Also discussed were effects of the TNF-family on tumor-associated cells, such as FasL-induced recruitment of the myeloid-derived suppressor cells (Qiuyan Liu), TNF-induced generation of IL10 in keratinocytes, which induces accumulation of macrophages with features characteristic of tumor-associated macrophages (Maria Ulvmar), and the TNF effect on generation of alarmins such as S100A8/A9 by endothelial cells, which in turn attract tumor cells, thus promoting metastasis (Yoshiro Maru).

2.3. Control of lymphocyte function and repertoire by the TNF-family

Effects of the TNF-family on all types of immune cells were discussed at the meeting. However, the major focus was on ways by which they affect lymphocytes. Two concomitant sessions were devoted to the latter subject. One session, chaired by Carl Ware and Jeff Browning, concentrated on the novel interactions and novel modes of cooperation, between interacting family members that widen their potential effects on lymphocyte function. Carl Ware discussed functional modulations of HVEM and its ligand LIGHT by the interaction of HVEM through a distinct interface with certain cellular Ig superfamily members (BTLA, CD160) and viral proteins (herpes virus envelope gD). He reported that BTLA–HVEM interaction prompts bi-directional signaling: activation of the canonical NF-κB pathway in epithelial cells expressing HVEM, and some pro-survival signals, as yet unknown, in T-lymphocytes expressing BTLA. Jennifer Gommerman presented evidence that LTαβ and CD40 ligand (CD40L) expression by $CD4^+$ T-lymphocytes differentially licenses dendritic cells to prime $CD8^+$ T-lymphocytes either for expansion or for effector functions. Taishin Akiyama discussed the cooperative effect of RANK and CD40L in inducing development of thymus epithelial cells in the adult mouse, and the expression by these cells (via activation of the alternative NF-κB pathway) of the protein Aire, which facilitates the promiscuous expression of peripheral tissue-specific antigens needed for deletion of autoreactive T-lymphocytes. Other presentations dealt with the ability of TNF to serve not only as a stimulator but also as a suppressor of T-lymphocyte function by binding to TNFR2 expressed by a subset of Treg cells and prompting, synergistically with IL2, the latter's expansion and expression of CD25 and Foxp3 (Daniela Männel); the ability of CD40L to boost autoimmunity by enhancing the expression of anti-apoptotic proteins in a CD40-expressing T-cell subset and by imposing "TCR revision" in these cells through induction of RAG1 and RAG2 (David Wagner); the ability of CD137 (4-1BB) to trigger reverse signaling by its ligand, thereby promoting survival and proliferation of hematopoietic progenitors as well as their differentiation to macrophages (Herbert Schwarz); distinct steps in the maturation of the lymph node anlage stroma for which LTβR signaling is required (Cecile Benezech); and the contribution of LTβR signaling to B-cell accumulation in the lacrimal gland of a mouse model of Sjögren's disease through induction of the chemokine Cxcl-13 (Roy Fava).

The concomitant session, chaired by Linda Burkly and Stephan Targan, focused on specific family members whose known contribution to immune regulation has recently been expanded. Participants discussed the functions of TWEAK and its receptor Fn14 in innate immunity (see above), as well as the emerging role of TL1A (TNFSF15) and its receptor DR3 (TNFRSF25) in T-cell-mediated autoimmunity, chronic mucosal inflammation, allergic lung inflammation, host defense against pathogens, and suppression of Treg function (Richard Siegel, Stephan Targan and Eckhard Podack). Both TWEAK and TL1A were proposed as potential targets for therapy of autoimmune diseases, particularly for chronic inflammation of the bowel. The powerful immune-stimulatory effect of TL1A was shown to be potentially useful in boosting vaccination. TL1A and APRIL were reported to be more effective than other TNF-family ligands as mucosal vaccine adjuvants (Mai Yoshikawa), and antibody-mediated triggering of DR3 was shown to significantly enhance antigen-specific CD8 CTL expansion (Eckhard Podack).

2.4. Functions of the TNF-family in infectious diseases

The primary role of immune defense is in combating infections. It is conceivable that this same role has also been the major determinant directing the evolution of the TNF-family, whose main function is to control immune defense. Interactions between the mechanisms of cellular response to pathogen components (now known to be mediated by the various pattern-recognition receptors (PRR)) and the generation and functions of TNF-family members have been evident ever since TNF was first described as a factor induced by bacterial endotoxin. A session on functions of the TNF-family in the context of infection, chaired by Tania Watts and Jürg Tschopp, began with introductions to major recent advances in the study of the molecular mechanisms activated by PRR. Luke O'Neill described negative feedback mechanisms that control signaling by the IL1/TLR family of receptors. Shigekazu Nagata presented new findings on signaling activation by the DNA of dying cells that has escaped degradation, and the mechanistic relationship of this signaling to the cellular response to intracellular foreign DNA. Jürg Tschopp described recently discovered ways in

which pathogen and host components activate processing of the inflammatory caspases by inflammasome complexes. Interestingly, signals induced by the T-cell-borne CD40 ligand and some other ligands of the TNF-family block activation of the inflammasome in macrophages, thereby restraining the innate immune response to pathogens.

The next group of presentations dealt with interactions of signals triggered by PRR with signaling-proteins activated by the TNF-family. Anna Yarilina showed that TNF, by activating IRF1, triggers chronic generation of IFN-β in macrophages. She further showed that the effects of IFN-β synergize with other TNF-induced signals, contributing to sustained inflammation, and priming the macrophages for enhanced production of type I interferons in response to adenovirus infection or stimulation of TLR7/TLR8 or TLR9. Akhil Rajput presented evidence that caspase-8, the proximal enzyme in death induction by the TNF-family, subdues innate responses to viruses by interacting with the RIG-like helicase complex (which mediates signaling in response to foreign ribonucleic acid). He further showed that in various tissues caspase-8 is needed in order to prevent spontaneous inflammation, which is apparently triggered by endogenous PRR ligands. Nikoletta Papadopoulou, Christine Chio and You-Sun Kim reported that TRADD participates not only in signaling by TNFR1 and DR3, but also in signaling by TLR3 and TLR4. The extent of its contribution was shown to vary in a cell-specific manner, apparently because of semi-redundancy with RIP1, whose expression level varies among different cells.

The second part of this session focused on the ways by which cellular responses to specific pathogens are affected by specific ligands of the family. Tania Watts presented evidence that GITR and 4-1BB both contribute, at different stages of influenza infection, to enhancement of the CD8 T-cell response to this virus. Nasiema Allie and Irene Garcia presented analyses of the protective effects of TNF against tuberculosis. They showed that such protection is mediated by the cell-bound form of the ligand, and that the expression of TNF by myeloid leukocytes is protective at early stages of infection. Raffaella Gozzellino showed that the necrotic damage seen in the livers of mice infected with *Plasmodium* occurs as a result of sensitization of their hepatocytes to TNF-induced toxicity by the large amounts of heme generated in digestion of hemoglobin by the pathogen. Joseli Lannes-Vieira presented evidence that the potentially fatal damage to heart tissue in *Trypanosoma cruzi*-induced Chagas disease depends largely on TNF function. Ali Alejo described a chimeric TNF/chemokine receptor that the poxvirus, ectromelia, generates to evade immune defense, and assessed the relative contributions of the two parts of those chimera to viral growth.

2.5. Functions of the TNF-family in neuronal development, physiology and pathology

The CNS, being "immune-privileged", is relatively well protected against the inflammatory and immune events activated by the TNF-family, and has therefore not been discussed to any great extent at the previous TNF conferences. Emerging evidence of pivotal contributions made by TNF and other family members to the development, physiology, and pathology of the CNS persuaded the conference's chairpersons to assign a session devoted to this subject at the current meeting. In the first presentation Tony Wyss-Coray, who co-chaired the session with Malú Tansey, set the stage for these discussions by reporting his recent discovery that the blood levels of several proteins involved in intercellular communication (including TNF and TRAIL receptor 4) undergo characteristic changes in patients with early-stage Alzheimer's, suggesting a link between the function of such proteins and the etiology of neurodegenerative diseases. As an example of a possible mechanism for this linkage he described studies in which TGF-β1 exhibited a dual impact on the CNS, exerting both neuroprotective and stimulatory effects on the accumulation of pathogenic T-lymphocytes that initiate autoimmune inflammation in the brain. Malú Tansey reviewed the literature documenting an association of microglial activation and overproduction of inflammatory mediators in the CNS with neurodegenerative disorders. She also presented evidence from her own studies suggesting that in patients with Parkinson's disease the death of nigral midbrain dopaminergic neurons, which are highly sensitive to TNF cytotoxicity, is induced by TNF. Era Taoufik reported that neurons deficient in caspase-8 are resistant to kainite-induced and NMDA-induced cell death, and that in mice with specific deletion of caspase-8 in CNS neurons kainic acid induces significantly less severe seizures and causes less neuronal death and less activation of caspase-3 than in the wild type. It is well known, however, that TNF also mediates neuroprotection, and Ulrich Eisel reported that TNFR2 was found to signal for neuroprotection through activation of protein kinase B/Akt and NF-κB. In line with that finding Mary Emmanouil reported that the protein kinase IKK2, which activates NF-κB, contributes to neuroinflammation at an early stage of experimental autoimmune encephalomyelitis (EAE) but is protective against axonal damage in the chronic phase. Yoshinori Takei also reported that TNF-mediated Akt activation enhances neuronal survival. He pointed out that NGF induces TNF synthesis in neuroblastoma cells, and that the TNF might, through Akt activation, interfere with the differentiation-promoting effect of NGF (which is ERK mediated) and contribute to tumor promotion. cFLIP, a protein known mainly for its role in protecting cells against death induced by the TNF-family, was reported by Rana Moubarak to bind to the tyrosine kinase NGF receptor TrkA and signal to neuronal differentiation through ERK and NF-κB activation.

An additional function of TNF, unique to the brain, was described by David Stellwagen, who showed that TNF release by glial cells is increased when neural activity is reduced, and that this enhanced release, combined with an ability of TNF to enhance excitatory synaptic strength (by causing exocytosis of AMPA-type glutamate receptors) and decrease inhibitory synaptic strength (by inducing endocytosis of GABA-A receptors), contributes to homeostatic synaptic plasticity.

Malú Tansey presented experimental evidence for the potential use of "dominant negative" TNF variants (see below) for blocking the neurodestructive effects of TNF without harming its neuroprotective and homeostatic functions. This was achieved by selectively inhibiting the function of soluble TNF while sparing that of the cell-bound form that differentially activates TNFR2.

Exciting new findings on the role of orphan member of the TNF/NGF receptor family, death receptor 6 (DR6), in neurodegeneration were presented by Anatoly Nikolaev. No ligand of the TNF-family that binds DR6 has yet been identified. Nikolaev found, however, that DR6 binds specifically to the soluble form of the amyloid precursor protein (APP) and is induced by it to activate a novel cell-death pathway that specifically affects the axons and is mediated by BAX and caspase-6, but is apparently independent of caspase-3. Nikolaev suggested that this axonal fragmentation process is induced upon trophic factor deprivation, which triggers β-secretase-mediated cleavage and hence shedding of the cell-bound form of APP. This mechanism of axonal fragmentation might contribute both to pruning of neurons in the course of normal neuronal development and to degenerative disease development.

3. New insights into molecular mechanisms

3.1. Cell-death mechanisms controlled by the TNF-family

The ability of TNF-family members to induce changes in cells that dictate their own demise has attracted attention ever since the

first known members (“lymphotoxins”) were discovered through this activity. The numerous reports presented on this subject demonstrated that despite the great progress made in clarifying the mechanisms of such cell-death induction, we still have a long way to go.

Peter Vandenabeele, who chaired this session together with John Eriksson, introduced the subject by describing the features and modes of induction of necrotic cell death. He focused on the fact that TNF can induce, besides caspase-mediated apoptotic cell death, a caspase-independent necrotic cell-death process that depends on the kinase function of RIP1 and on mitochondrial complex I and PLA2 activities. Applying siRNA libraries to identify genes contributing to this process, Xiaodang Wang, as well as Francis Chan, found that in addition to RIP1, this necrotic pathway depends on the homologous kinase RIP3. Induction of necrosis by TNF, FasL, or TRAIL is initiated by induced association and phosphorylation of these two proteins in a way that depends on the kinase activity of both (that of RIP3 apparently having the initiating role). Variation of the cellular levels of RIP3 largely determines the ability of these ligands to induce necrosis. Its deficiency provides protection from necrotic tissue damage, but also impairs a defensive role of the necrotic process in situations such as viral infections.

While the exact mechanism of the necrotic effect is still unknown, it seems to involve ROS generation, either by TNF receptor associated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or by the mitochondria. A potential proximal component of a TNF-induced ROS-dependent cell-death pathway was presented by Martin Krönke, who showed that activation of NADPH oxidase by TNF depends on the recruitment of a novel riboflavin kinase to the TNF receptor complex. Zhenggan Liu, on the other hand, described a novel protein, ATIA (anti-TNF-induced apoptosis), which translocates from the plasma membrane to the mitochondria where it associates with a specific target protein, thereby allowing TNF to induce necrotic death while arresting its induction of apoptotic death.

A glimpse into an additional TNF-family induced cell-death pathway that apparently also involves mitochondrial function was provided by Masayuki Miura. Presenting his recent findings of the cell-death mechanism triggered by Wenger and Eiger, the *Drosophila* members of the TNF ligand and receptor families, he reported that this death, previously shown to involve activation of c-jun N-terminal kinase (JNK) through the function of the *Drosophila* homologues of TRAF2 and TAK1 (DTRAF2 and dTAK1), also depends on a group of genes involved in mitochondrial energy production.

New knowledge was also presented on the caspase-dependent apoptotic pathway activated by ligands of the family. Avi Ashkenazi reported that effective activation of caspase-8 requires its ubiquitination within membrane rafts to which it translocates following generation of death-inducing signaling complex (DISC), and its further aggregation by the binding of its linked polyubiquitin moieties to the ubiquitin-binding protein p62. Compartmentalization of caspase-8 in death induction was also reported by Uwe Bertsch, who described translocation of the enzyme to endocytic vesicles in association with the TNF receptor complex (“receptosome”), followed by caspase-8-mediated activation of caspase-7 and then by caspase-7-mediated activation of pro-acid sphingomyelinase.

Another major focus of this session was on the mechanisms underlying the “decisions” about whether a particular ligand will induce cell death or a non-deadly effect in a given situation, and if the former, whether the death will be apoptotic or necrotic. Prior studies have shown that these “decisions” are affected by molecular determinants that act on numerous mechanistic levels, and the findings provided further insight into some of them. Roles of molecular determinants that interact with the ligand protein in determining the kind of effects that this ligand induces were exemplified by several presentations. The differences in functions of the soluble and the cell-bound forms of TNF imply that molecular determinants affecting TNF shedding may determine the outcome of TNF function. FasL function is controlled in a similar manner. Bruce Ksander and Meredith Gregory reported that retinal ganglion cell death is induced by FasL only when the latter is bound to the cell membrane, and that the TNF-induced enhanced generation of the cell-bound form of FasL by ocular tissues is a major cause of the retinal ganglion cell loss associated with glaucoma and other optic neuropathies. Generation of exosomes (shed membrane vesicles) whose membranes present bound FasL greatly enhances the exposure of target cells to this ligand. Sophia Cleland reported that exosome shedding by T-lymphocytes requires the function of Wiskott–Aldrich syndrome protein. Deficiency of this protein significantly decreased re-activation-induced death of the T-lymphocytes, resulting in autoimmunity. Another distinctive feature of the cell-bound form of FasL was presented by Martin Zörnig, who showed that the ligand’s intracellular domain acts as a nuclear repressor in lymphocytes, suppressing signaling for activation-induced proliferation.

The spatial aspect of the dynamics of the signaling activated by the TNF-family—the fact that signaling complexes can become modified and restructured as they move along their signaling route so that a distinct signaling output is generated at each compartment—was discussed in connection with signaling for other functions of this ligand family as well. A similar spatial aspect was also shown to apply to signaling activated by PRR. This spatial aspect allows for additional levels of signaling regulation, which are specifically linked to the control of subcellular localization of the signaling complexes.

In addressing the variety of mechanisms that control death induction, several presentations focused on the extent to which death resulting from signaling for a given receptor of the TNF/NGF family is dependent on inputs provided by additional receptors. Lester Lau reported that triggering of integrins and heparan sulfate proteoglycans by CCN1, a matrix protein up-regulated at sites of inflammation and wound repair, induces ROS and JNK activation, which directs TNF-induced signaling for death. Martin Ehrenschwender reported that death induction by TNF is also potentiated via co-stimulation of Fn14 by TWEAK, apparently due to TWEAK-induced depletion of TRAF2. Conversely, signaling for death by the TRAIL receptors DR4 and DR5 is blocked by triggering of the TRAIL decoy receptor DcR2. Najoua Lalaoui reported that even though DcR2 lacks an intracellular domain, its inhibitory effect on death induction is exerted by intracellular signals (activation of the PI3K/Akt pathway) that this receptor generates.

Also described were a number of proximal events in the signaling by death receptors that affect the “death versus life” decision. Adrian Ting suggested that in T cells the ubiquitination of RIP1 is a major determinant of this decision. Ubiquitinated RIP1 can bind NEMO and thus recruit the IKK signalosome, resulting in NF-κB activation, with consequent generation of anti-apoptotic proteins. Ubiquitination also interferes with the binding of RIP1 to caspase-8, which is needed for effective caspase-8 processing, and it therefore interferes with induction of apoptotic death even when anti-apoptotic protein synthesis is blocked. Since RIP1 also signals for necrotic death (in a way that depends on its protein kinase function), apoptotic death induction might be facilitated through the eventual cleavage of RIP by caspases. Inhibition of caspase activity by agents like zVAD may indeed transform the type of death induced by TNF to necrosis. Findings presented by Han-Ming Shen indicated that death induced in the presence of caspase inhibitors such as zVAD is also affected by this agent’s ability to induce generation of TNF through activation of the alternative NF-

κB pathway. TRAF2 and the cIAP proteins are known to suppress death induction by the TNF receptor. John Silke presented evidence that this function is crucially dependent on association of the cIAPs with a specific binding surface in TRAF2 as well as on the latter's ubiquitin ligase activity (though his data suggest that this ligase activity is not required for NF-κB activation). A role not less important in suppressing early signaling for death by the death receptors is served by the various splice variants of cFLIP. John Eriksson addressed the importance of signals that prompt ubiquitination of cFLIP in determining the vulnerability of cells to death induction. He reported that phosphorylation of a specific serine residue (S193) in s-FLIP dictates ubiquitination and degradation of the short isoforms of this protein (S and R) but not of the full-length one (c-FLIP-L), and that this phosphorylation is operated by PKC (mainly PKC-α and PKC-β). Philipp Jost addressed the post-receptor role of XIAP in determining cell vulnerability to death induction. He showed that in hepatocytes, the presence of XIAP withholds mitochondria-mediated potentiation of TNF-induced death.

In sharp contrast to the prodigious increase in information on the mechanisms mediating and regulating death induction by ligands of the TNF-family, our knowledge of the actual physiological and pathological roles of this death activity and their relative significance is rather limited, and is indeed much poorer than that of many other functions of these ligands. In view of this limitation, there was particular interest in the findings presented by Lisa Sedger that FasL and TRAIL together serve a crucial role in the activation-induced death of T-lymphocytes. Deletion of the two ligands together in mice therefore results in a far more severe lymphoproliferative disease than that observed in mice deficient in FasL alone.

3.2. Functional roles of ubiquitin ligases and different modes of ubiquitination in signaling initiation by the TNF-family

Another major focus of discussion was the contribution of signaling-protein ubiquitination to the initiation and the cessation of signaling by the TNF-family. The structural and functional versatility of ubiquitin chains—the different ways in which ubiquitin moieties are linked to proteins or linked to one another within polyubiquitin chains conjugated to proteins—and the fact that these various modes of linkage may dictate the same functional consequences in some situations and different functional consequences in others, were introduced by Aharon Ciechanover. In the case of some proteins, linkage of multiple monomeric ubiquitin molecules dictates their uptake to lysosomes. In the case of the p105 NF-κB precursor protein, however, such ubiquitination was shown by Ciechanover to dictate limited proteasomal processing that yields the mature p50 NF-κB subunit. He also reported that the ubiquitin B+1 (UBB+1) protein, a molecular misreading product of the UBB gene, which accumulates in affected cells in various neurodegenerative diseases (e.g. Alzheimer's), is a poor substrate for the proteasome chiefly because of its limited length. Its extension by only six amino acids converts it to a normally metabolized polypeptide, demonstrating the length dependence of proteasomal degradation.

The impact of ubiquitination on specific signaling pathways activated by the TNF/NGF family of receptors was discussed in a session chaired by John Silke and Domagoj Vucic that was devoted to the TRAFs and the cIAPs, the two main groups of E3 ubiquitin ligases known to be recruited to this receptor family, as well as in a session chaired by Daniela Männel and David Wallach that covered more general aspects of signaling by these receptors. Two main questions were addressed in connection with the impact of ubiquitination on all of the signaling pathways activated by the TNF-family. First, what changes in protein interactions are imposed by the ubiquitination? Secondly, how is this impact affecting—and being affected by—a protein's subcellular localization? Particular attention was directed to the question of substrate and linkage specificity of E3s. Is it complex dependent and modification dependent, and can it really be altered during the signaling process? In all the signaling pathways discussed, canonical ubiquitination (K48-linked) was reported to impose degradation of signaling-proteins and, as a consequence, dissociation of the complexes of which they form part, whereas non-canonical ubiquitination (e.g. K63-linked or linkage of head-to-tail linear chains) reportedly dictated association of proteins or modification of their activity. The ways in which these ubiquitination effects contribute to those processes were shown, however, to differ for the different signaling pathways.

As indicated in several presentations, in response to stimulation of TNF/NGF family receptors the activation of IKK2, the protein kinase that initiates the canonical NF-κB pathway, is triggered by recruitment of the IKK complex (comprising IKK1, IKK2 and NEMO) to the receptor complex. The recruitment seems to be driven by association of a distinct ubiquitin-binding motif in NEMO ("Ubiquitin Binding in ABIN and NEMO"; UBAN) with non-canonical ubiquitination chains that are linked to one or more signaling-proteins associated with the receptors. Degradation of the receptor-associated protein to which NEMO binds is induced by that protein's K48-linked ubiquitination, allowing release of the IKK complex to the cytoplasm.

Findings presented by Michael Karin and Ewen Gallagher indicated that triggering of the MAPK pathways by TNF-family ligands through activation of MAP3Ks such as MEKK1 is also initiated by recruitment of the protein kinases to the receptor, followed by their release to the cytoplasm. In these cases too, the release is facilitated by protein degradation dictated by K48-linked ubiquitination. Activation of MAP3Ks occurs, however, only after their release by the receptor to the cytoplasm. The protein whose degradation allows this release is TRAF3. Its degradation is driven by the proteins's K48-linked ubiquitination, catalyzed by cIAP1 or cIAP2 (cIAP1/2) that bind to TRAF2 (which in turn binds TRAF3).

As described by Karin, activation of the protein kinase NIK, which initiates the alternative NF-κB pathway, also requires degradation of TRAF3 (as well as of TRAF2) following the ubiquitination of these two proteins by cIAP1/2. In that case, however, the signaling complex that dissociates as a result of this degradation already existed prior to stimulation. Vucic reported that in this pre-existing complex cIAP1/2 acts to dictate the ubiquitination and degradation of NIK itself, and the breakdown of the complex results in arrest of NIK ubiquitination, with a consequent increase in its cellular amount. The two cIAPs act redundantly in this process and, as reported by Techno Tenev, the cellular level of cIAP2 is down-regulated by cIAP1, a process that serves to buffer the ubiquitin ligase activity shared by these two proteins. Once TRAF2 is recruited to the receptor complex, its non-canonical ubiquitation of the cIAPs redirects their ubiquitin ligase activity from NIK to the TRAFs.

We do not yet know the exact subcellular site at which NIK and the alternative NF-κB pathway are activated. However, as reported by Emmanuel Dejardin, at least in the case of activation of this pathway by LTβR, this site is distinct from the site at which the canonical pathway is activated: whereas the alternative pathway is activated in an intracellular compartment to which the receptor complex is directed in a clathrin-independent way, clathrin-mediated endocytosis is required for activation of the canonical pathway.

Studies presenting a more detailed scrutiny of the mechanism by which TNF activates the canonical pathway raised some questions: (a) what is the nature of the ubiquitin chains with which the ubiquitin-binding domain in NEMO associates? The currently

prevailing notion is that the NEMO-binding chains are K63-linked. Crystallographic analysis presented by Fumiyo Ikeda indicated, however, that *in vitro* this domain binds exclusively to linear (head-to-tail) diubiquitin, whereas data presented by Hau Wu suggested that it can bind both types of chains, though the K63-linked poly-Ub chains required for interaction with NEMO might be longer. Wu further showed that each ubiquitin in the diubiquitin molecule interacts with the symmetrical NEMO domain asymmetrically, through linkage-specific binding surfaces. (b) What are the receptor-associated proteins to which the ubiquitin chains that bind NEMO are linked? Published data suggest that in the TNF receptor complex the protein to which these ubiquitin chains are linked is RIP1. It seems, however, that RIP1 does not associate with every one of the TNF/NGF receptors that activates NF-κB by recruiting the IKK signalosome. Moreover, as reported by John Silke, in MEFs deficient in RIP1, TNFR1 is still capable of activating NF-κB, and he suggested that the TNF receptor complex contains some redundant proteins to which the NEMO-binding ubiquitin chains are linked. (c) What is the ubiquitin ligase that generates the NEMO-binding polyubiquitin chains? Several published studies suggest that this ubiquitination is catalyzed by the RING finger of TRAF2. John Silke reported, however, that a TRAF2 mutant deficient in active RING finger can effectively restore RIP1 recruitment and ubiquitination as well as NF-κB activation in TRAF2-deficient cells, and Domagoj Vucic showed that non-canonical ubiquitination of RIP1 can be mediated by cIAP1/2 (although the E2 in this case is apparently not Ubc13/Uev1A as in the case of ubiquitin ligases known to mediate K63-linked ubiquitination, but rather UbcH5a). Henning Walczak reported that the proteins HOIL-1 and HOIP, which together form a linear ubiquitin chain assembly complex (LUBAC), are recruited to the TNFR1 complex, where they dictate linkage of linear ubiquitin chains to proteins within this complex. Such ubiquitination does not seem to be crucial for signaling initiation, but it strongly influences the extent of NF-κB and JNK activation and the resulting gene induction.

Although the downstream signaling effects of TRAF6 are quite similar to those of TRAF2 and TRAF5, its functional roles and the mechanisms by which TRAF6 initiates these signaling pathways are not. Crystal structure analysis of TRAF6, reported by Hao Wu, provided a glimpse of the structural basis for these features, in particular for the potent K63-linked ubiquitination that TRAF6 catalyzes. This activity was found to depend on the formation of dimeric complexes of TRAF6 with its E2 (Ubc13), and this in turn induces higher-order TRAF6 oligomerization, forming a large molecular platform suggested to promote polyubiquitin synthesis, auto-ubiquitination, and recruitment of downstream proteins.

The sessions dealing with signaling-protein ubiquitination provoked lively round-table discussion. All agreed that while the knowledge gained of the ubiquitination mechanisms that initiate signaling activation has significantly advanced our understanding of these processes, major gaps still remain. In particular, despite having quite substantial information about protein associations, dissociations, and translocations that dictate activation of the proximal protein kinases in the signaling pathway, we still have no clear knowledge of the structural and biochemical basis for the activation itself.

Identification of the ubiquitin ligase activities that initiate the processes provides some insight into additional mechanistic levels at which signaling can be modulated. An example of such modulation, described by Vishva Dixit, is "ubiquitin editing" by A20, a protein that serves as a negative feedback inhibitor of signaling initiation by inducers of inflammation. It does this by first prompting degradation of non-canonical ubiquitin chains linked to a signaling-protein such as RIP1, and then inducing degradation of the signaling-protein itself by catalyzing its canonical ubiquitination. Geert van Loo described a study of the conditional knockout of A20, which further confirmed that the negative impact of this protein on signaling initiation is crucial for preventing severe inflammatory processes. Two other examples of proteins that regulate signaling by the TNF-family through their effects on ubiquitination of signaling-proteins concerned the canonical and non-canonical ubiquitination of TRAF2. Anning Lin described a novel protein, SMOR1, which is constitutively associated with TRAF2 and JNK1 in non-stimulated cells and prevents the spontaneous initiation of K63-linked auto- and hetero-ubiquitination by TRAF2. SMOR1 possesses ubiquitin ligase activity, which enables it to induce its own canonical ubiquitination and degradation after TNF treatment. Its degradation unleashes the non-canonical ubiquitin ligase activity of TRAF2, resulting in activation of the JNK (but not the p38 or the NF-κB) pathway. The K48-mediated ubiquitination of TRAF2 itself was reported to be regulated by TRAF1. As the sole member of the TRAF family that is deficient in the ubiquitin ligase RING finger motif, TRAF1 apparently serves a number of different functions. Tania Watts reported that recruitment of TRAF1 to 4-1BB is required for the activation of ERK by this receptor, which in turn signals for suppression of BIM expression in T-lymphocytes, thus prolonging their survival after antigen withdrawal. In addition, however, Andreas Wicovsky reported that TRAF1 also protects cells from the cytotoxic effect of TNF and enhances TNF-induced inflammation, and that it does so by blocking the TNFR2-induced K48-linked ubiquitination of TRAF2 (which is probably mediated by cIAP1/2), thereby enhancing signaling for JNK and NF-κB activation. The same group reported that TRAF1 inhibits LTαβ2- and TWEAK-induced translocation of TRAF2 to a cellular compartment that is resistant to detergent, and that this effect correlates with enhanced activation of the canonical NF-κB pathway by these ligands.

4. Application of our knowledge to therapy

As reviewed in the plenary lecture by Ravinder Maini, blocking of TNF by injection of recombinant soluble TNF receptors or anti-TNF antibodies has by now been carried out in over a million patients, with long-term benefits in chronic inflammatory conditions like rheumatoid arthritis (RA), psoriasis, and the inflammatory bowel diseases. This recombinant protein-based therapy is expensive, and so is still largely restricted to the more affluent countries. Moreover, not all patients benefit from such treatments. (In patients with RA, for example, 70% are initial responders; about 50% are still responding after 5–10 years, and ultimately 40% to 50% may need other drugs.) There is also concern that, apart from compromising the immune response to certain pathogens such as tuberculosis, blocking of TNF might have some other adverse effects in certain patients, such as skin or demyelinating diseases and some kinds of cancer. It is not yet understood what determines whether a given patient suffering from a disease that appears to depend on TNF will benefit from anti-TNF therapy, fail to respond, or—as happens in rare cases—develop adverse effects.

A similar gap between our knowledge of TNF function and its translational implications was discussed by Frances Balkwill in connection with the potential therapeutic effect of TNF itself in cancer. TNF appeared to play a major role in the anti-cancer effects reported more than a century ago by Coley in his attempts to treat cancer patients with bacterial toxins. In animal models, moreover, TNF caused selective and effective destruction of tumors. When applied to humans, however, treatment with TNF was found to also yield life-threatening effects, and further trials were discontinued.

The finding that both blocking and enhancing of TNF activity can have beneficial effects but can also be detrimental still challenges our understanding of the way this cytokine acts. It also impels us to explore the possible application of blocking and

enhancing the activities of other TNF-family members family for therapy. First attempts at such exploration were described by Jeffrey Browning, who described the initial outcomes of trials in which therapy with LTβR decoy was tested, and by Avi Ashkenazi, who provided a brief update of attempts to use the TRAIL ligand in cancer therapy.

Because of the extraordinary importance of this subject, the conference chairpersons decided that despite the heavy time pressure more than half a day would be set aside for intensive discussions aimed at strengthening the scientific basis for defining effective therapeutic approaches. A plenary session, chaired by Marc Feldmann and Ravinder Maini, was followed by a workshop, chaired by Ravinder Maini and Claude Libert, on TNF-family-related effects that might account for pathological consequences of inducing—or of blocking—ligand functions. A concomitant session, chaired by Klaus Pfizenmaier and David Szymkowski, focused on the rational design of agents aimed at achieving better therapy, again either by triggering effects of the ligands or by blocking them.

4.1. Mechanisms underlying pathological and therapeutic effects of the TNF-family

One of the main conclusions derived from the session described below was that despite the multiplicity of potentially deleterious cellular effects of TNF, it is possible to identify a restricted set of specific effects that play pivotal roles in given pathological situations. This set might vary, however, depending on the affected tissue. Claude Libert reported that two effects of TNF are pivotal to the systemic inflammatory response syndrome, a fatal disease in mice exposed to high levels of this cytokine. One is the activation of matrix metalloproteinases (blockable by specific inhibitors), and the other is the arrest of the protective activity of glucocorticoids, which results from the decrease in stability of the corticoid receptor in response to TNF. (The latter effect can be antagonized by orally ingested $ZnSO_4$, which protects the glucocorticoid receptor by enhancing the expression of HSP70.) Mathur Kannan suggested that airway hyperresponsiveness induced by TNF is largely attributable to the induction of CD38 in the smooth-muscle cells of the airway. Andrew Cope suggested that the deficient T-cell responsiveness observed in patients with RA results from the TNF-induced increase of IκBε, which blocks nuclear translocation of c-Rel and thus inhibits IL2 induction. Dewan Majid reported that renal injury caused by TNF is a result of induced vasoconstriction and hypofiltration, which appear to be mediated by enhanced ROS generation. Ravinder Maini reported that in studies of RA patients treated with TNF blockers the earliest functional impact of TNF inhibition was arrest of inflammatory cell recruitment, suggesting that the effect of TNF on this recruitment makes a pivotal contribution to the pathology of RA. A presentation by the group of Michael McDermott revealed a significant difference between the impacts of TNF inhibition on the circulating leukocyte patterns in early and late RA, which might be relevant to the differences in clinical response to such inhibition in these disease states. In discussing the mechanisms by which TNF facilitates inflammatory bowel diseases, Claude Libert reported that the TNF-induced pathology of the small intestine seems to involve a key role of IL17 released from stores in the Paneth cells. Katerina Vlantis showed, by assessing the effect of NEMO deficiency in the intestinal epithelium, that when activation of the canonical NF-κB pathway in these cells is arrested, endogenously produced TNF induces intestinal inflammation by activating the extrinsic cell-death pathway, causing epithelial cell death. Another tissue that is vulnerable to the cytotoxic effects of both TNF and FasL is the liver. While death of hepatocytes results from mere exposure to FasL, their killing by TNF requires the additional effect of agents that augment JNK activity with consequent generation of ROS, thereby sensitizing the cells to the TNF onslaught. As mentioned in Section 2.4, similar sensitization is observed in response to the heme that accumulates during *Plasmodium* infection (Raffaella Gozzellino). Jörn Schattenberg and Hiroyasu Nakano reported that heightened JNK activity also accounts for the increased vulnerability to TNF and FasL cytotoxicity of hepatocytes deficient in cFLIP. In exploring the mechanisms underlying sensitization of the liver to TNF toxicity (when caused artificially by galactosamine), Thomas Kaufmann found that such sensitization also occurs through JNK activation and depends on the induction of the BH3-only Bcl2 protein, BIM. As opposed to FasL and TNF, TRAIL is not known to be toxic to hepatocytes. Many other normal cells are also resistant to the cytotoxicity of this ligand. However, according to a report by Ko Okumura, the epithelial cells of the bile duct (cholangiocytes) are sensitive to the cytotoxic effect signaled by the TRAIL receptor DR5, to a variable extent that depends on genetic background and environmental determinants. This finding raises the possibility that TRAIL cytotoxicity contributes to chronic cholestatic disease and emphasizes the need for suitable precautions when applying TRAIL for tumor therapy.

Studies of mice with tissue-specific knockout of TNF in T cells or macrophages showed that in different cell types distinct TNF functions can inflict pathological changes (Sergei Nedospasov). For example, deletion of TNF in macrophages and neutrophils resulted in increased numbers of autoreactive CD4 T cells in mice with autoimmune encephalitis or collagen-induced arthritis, indicating that TNF expression in macrophages may control early expansion of autoimmune T cells. Findings presented by Dirk Elewaut raised the possibility, however, that at least part of the various functional aberrations contributing to TNF-induced pathologies occur secondarily to a common initiating event. The latter findings suggested that several diseases to which TNF contributes (spondyloarthritis, RA, inflammatory bowel diseases), although eventually involving multiple cell types, may have a common cellular pathway initiated by TNF-induced effects in mesenchymal cells. Elewaut also presented findings suggesting that all of these different pathologies are negatively regulated by TNF-mediated activation of inflammatory dendritic cells, which in turn activate invariant NKT cells.

In discussions of the harm that can be caused by anti-TNF therapies that also arrest beneficial TNF activities, a major issue raised was the apparent preferential involvement of the soluble TNF in causing such harm. The more innocuous character of cell-bound form might be attributable in part to the differential ability of this form to activate TNFR2. David Goukassian reported that triggering by TNF of this receptor was found to be crucial for repair and regeneration of the myocardium after acute infarction, apparently reflecting functional roles of this receptor both in the heart tissue and in bone-marrow-derived endothelial progenitor cells. As mentioned in Section 2.5, TNFR2 also mediates beneficial effects of TNF on neurons in the brain (Ulrich Eisel).

4.2. Rational design of new therapeutic modulations of TNF-family functions

As illustrated by Yosef Yarden from the experience gained in attempts to develop anti-cancer drugs based on inhibition of EGF receptor signaling (Section 1.2), a signaling pathway that leads to disease may offer a variety of potential targets for therapy. The wide range of subjects covered in the workshop and poster sessions about rational design of new therapies reflects the multiplicity of mechanistic levels, as well as the heterogeneity of types of modulation, by which scientists seek to exploit functions of the TNF-family for therapeutic purposes. Most of the attention in these sessions was directed to manipulation of ligand–receptor interactions with the aim of achieving therapeutic modulation of signaling.

A number of presentations addressed the potential advantage of blocking deleterious (proinflammatory) effects of the soluble form of TNF (which activates TNFR1) while maintaining the beneficial effects (induction of innate immunity) of the cell-bound form (which also activates TNFR2). David Szymkowski described a "dominant negative" TNF mutant devoid of signaling function or ability to associate with the cell-bound form of TNF and yet still capable of associating with the soluble form, generating inactive TNF trimers. He described its use in the selective blocking of functions mediated by the soluble form. Yasuo Tsutsumi described an alternative means to a similar end, by employing a mutant of TNF that binds selectively to TNFR1 but is incapable of triggering its signaling, and thus acts as a selective inhibitor of this receptor. When its serum half-life time was increased by its linkage to polyethylene glycol, this TNF antagonist was highly effective in inflammatory disease models and exhibited a lower inhibitory effect on TNF-mediated immune defense than agents which blocked TNF binding to both receptors. Other presentations described sophisticated screening approaches, based on phage display and advanced design algorithms, to identify mutants of ligands with opposite properties to the above, namely enhanced ability to trigger signaling (TNF and LT, discussed by Tsutsumi) or ability to selectively trigger one but not the other receptor of a given ligand (TNFR1- and TNFR2-specific TNF variants, Tsutsumi; DR5- and DR4-selective human TRAIL variants, Wim Quax).

Generation of therapeutically useful mutants of ligands and soluble receptors, even when done by screening of randomly mutagenized proteins, is based on the findings of basic studies of structure-function relationships in these proteins. Examples of insights gained from such basic studies were presented by a number of scientists. Pascal Schneider reported that deletion analysis of EDA1 revealed that its collagen domain, which is cleaved off in shedding of this ligand, functions as an oligomerization unit, and is therefore required for its effective signaling, while a stretch of basic amino acids located N-terminally to the collagen domain has proteoglycan-binding ability that apparently restricts the distribution of endogenous EDA1 *in vivo*. Anja Krippner-Heidenreich showed that the differential dependence of TNFR2 on triggering by the cell-bound form, as well as the different extents of ligand-independent receptor assembly of the two TNF receptors, are dictated by the stalk and the first cysteine-rich domain regions in the receptors. Marcos Milla reported that the prodomain of TACE, the enzyme that mediates shedding of TNF and its receptors, contains short regions that function to maintain the enzyme in a non-active state in the absence of appropriate stimulation.

Some uses of new kinds of antibody preparations were also presented. Andreas Evdokiou described a fully humanized anti-DR5 antibody (Apomab) that effectively destroys tumors in mouse breast cancer models. Jan Paul Medema described a new antagonistic anti-APRIL antibody and its potential use for therapy of B-cell malignancies in which APRIL apparently functions as a pro-survival factor, as well as for therapy of autoimmune diseases.

Other presentations dealt with attempts to synthesize organic molecules that can serve as therapeutic substitutes for natural ligands and receptors or antibodies against them. Stephen Hale described the development of small macrocyclic molecules that inhibit TNF binding to its receptors and show anti-inflammatory activity *in vivo*. Lih-Ling Lin described the development of inhibitors of the MAP3K Tpl2. These inhibitors were found to block LPS- and IL1-induced TNF production and reduce disease severity in the murine collagen-induced arthritis model. Since this kinase has a rather restricted set of functions, it is hoped that such inhibitors will prove suitable for anti-TNF therapy. Examples of compounds that affect TNF-activated signals and might well turn out to have therapeutic effects were presented by several of the speakers. In the session on TRAFs and cIAPs, speakers discussed the therapeutic potential of small-molecule peptide mimetics of the mitochondrial IAP-inhibitory protein SMAC for inducing auto-ubiquitination and degradation of cIAP1/2. According to their reports, such compounds might serve as effective anti-cancer agents by sensitizing cancer cells to the apoptotic effects of TNF-family ligands, and in some cell types triggering death even in the absence of such ligands by inducing TNF synthesis, as well as by inducing generation of a caspase-8-activating complex consisting of RIP1, FADD, and caspase-8. (Domagoj Vucic, John Silke, Xiaodong Wang).

Besides describing new kinds of therapeutic reagents, speakers also provided several examples of new kinds of cellular effects whose induction by such reagents might serve therapeutic purposes. Jane Grogan showed that antibodies to LTα, by binding to Th1 and Th17 T-lymphocytes which express LTα1β2, can induce Fcγ-receptor-mediated immunoablation of these T-cell subsets, thereby ameliorating autoimmune diseases in mouse models. Klaus Pfizenmaier described the generation of bioactive single-chain TRAIL and its further fusion with a single-chain antibody against the EGF receptor family member ErB2 which, by targeting TRAIL toxicity to Erb2-expressing cells, facilitates their selective destruction. Marjaneh Razmara described the generation of soluble chimeras of TRAIL and Fn14 which, when expressed by transfected DNA *in vivo*, attenuated EAE in mice (apparently by combined TRAIL-mediated killing of activated T-lymphocytes and blocking of the proinflammatory effects of TWEAK) to a much greater extent than combined treatment with TRAIL and Fn14.

Why do different people with an apparently identical disease sometimes respond differently to a given therapeutic tool? In an inspirational talk on the application of a systems approach to medicine, Leroy Hood described the potential of this approach, when combined with technologies of DNA sequencing, protein profiling and computation, to transform medicine "from its currently reactive state to a mode that is predictive, personalized, preventive and participatory." Further refinement of these technologies, together with more detailed knowledge of patients' genetic differences, can be expected to facilitate earlier detection and more precise classification of diseases. In addition, it is likely to yield better understanding of the reasons why a particular therapeutic approach can elicit heterogeneous responses. It is to be hoped that it will also allow us to design better therapies.

5. Facilitating communication within the expanding superfamily of scientists who study the TNF superfamily

The ongoing proliferation of knowledge about the TNF-family is reflected in a prolific increase in the number of scientists who contribute to the studies in this field. In hundreds of laboratories throughout the world there is intensive exploration of the basic, medical, and translational issues in connection with the functions of this family. The range of tangentially related fields on which studies of the TNF-family have important bearings has also undergone remarkable expansion.

At the present TNF conference, some changes in the traditional design of the meeting were introduced in the interest of adjusting to this expansion. The aim was to refresh and extend the circle of participants who attend and who present their work, and to encourage interaction among scientists through discussion of issues specific to the field as well as their implications for related fields. Most sessions began with a talk by an eminent guest lecturer on a subject tangentially related to an aspect of the TNF field to be addressed in that session, and most ended with a round-table discussion based on outlines defined by the session's moderator(s) and posted in advance on the meeting's website.

The conference was very well attended. There were numerous presentations of interesting findings, and it was sometimes necessary to hold two sessions in parallel. Several topics were

addressed intensively for the first time by having specific workshops devoted to them. These topics included signaling networks and systems biology, gene regulatory networks and miRNAs, mechanisms of pathological and therapeutic effects of the TNF-family, rational design of new tools for therapeutic modulation of TNF-family functions, and roles of the TNF-family in neuronal development, physiology and pathology. To accommodate larger numbers and varieties of presentations, most of the talks were rather short. However, the presented summaries of most talks in posters that were displayed throughout the meeting, as well as the round-table discussions at which questions raised in the talks could be thrashed out, made it possible to compensate for the time limitations.

There are no geographic limits to the proliferation of laboratories devoted to TNF research, and important contributions to the field are emerging from scientists all over the globe. Following the previous meeting held two years ago in California, choosing Spain for the present conference was in line with the tradition of shifting the location to allow students from different countries to attend without always having to cope with the expense of long flights. For quite a few years, however, all meetings have taken place in Europe or the USA. We are happy to tell you that the next TNF conference will be held on May 15–18, 2011, at the Awajishima Conference Center on Awaji Island, Japan. It will be chaired by Shigekazu Nagata and Masayuki Miura, with the assistance of a local advisory committee of scientists from different countries in Oceania.

Mark your calendars now!

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David Wallach obtained his B.Sc., M.Sc., and Ph.D. degrees from the Department of Biological Chemistry, The Hebrew University of Jerusalem, Israel. He did his M.Sc. research with Dr. Izak Ohad and his doctoral research with Dr. Michael Schramm. After completing his doctoral studies in 1974, he spent three years as a visiting fellow at the National Cancer Institute (National Institutes of Health, Bethesda, MD). While there, he did postdoctoral research in the laboratory of Dr. Ira Pastan. In 1977 he returned to Israel, where he was appointed fellow in the Department of Virology at the Weizmann Institute of Science in Rehovot. His studies during that period were the first to provide conclusive evidence that the 'type I' and 'type II' interferons act through distinct mechanisms and have distinct patterns of effects. He was appointed associate professor at the Weizmann Institute in 1983 and a full professor in 1995. He is currently working in the Institute's Department of Biological Chemistry. Over the past 25 years, Prof. Wallach and his colleagues have been engaged in elucidating the mode of action of cytokines of the TNF-family. They did pioneering work in isolating TNF, isolating and cloning the soluble and cell-surface forms of the TNF receptors and exploring their shedding mechanisms, and cloning the major components of the extrinsic cell-death pathway (FADD/MORT1, caspase-8/MACH, and cFLIP/CASH) and exploring their mechanisms of action. They cloned NIK (the protein kinase initiating the alternative NF-κB pathway), discovered that activation of the canonical NF-κB pathway by TNF is associated with covalent modification of RIP1 and recruitment of NEMO to it, and cloned CYLD, a K63-specific deubiquitinase that acts as a negative feedback regulator of signaling.

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