Therapeutic Targets of the TNF Superfamily

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Volume 647
THERAPEUTIC TARGETS OF THE TNF SUPERFAMILY
Edited by Iqbal Grewal

Therapeutic Targets of the TNF Superfamily

Edited by

Iqbal S. Grewal, PhD

Department of Preclinical Therapeutics, Seattle Genetics, Inc., Bothell, Washington, USA

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Phone: 512/ 637 6050; FAX: 512/ 637 6079
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PREFACE

Tumor necrosis factor (TNF) superfamily is a rapidly growing family of cytokines that interacts with a corresponding superfamily of receptors. Ligand-receptor interactions of this superfamily are involved in numerous biological processes ranging from hematopoiesis to pleiotropic cellular responses, including activation, proliferation, differentiation, and apoptosis. The particular response depends on the receptor, the cell type, and the concurrent signals received by the cell. Worldwide interest in the TNF field surged dramatically early in 1984 with the cloning and defining of the profound cellular effects of the first member of this family, TNFα. Subsequently, the major influence of TNFα on the development and functioning of the immune system was established. Today, over 20 human TNF ligands and their more than 30 corresponding receptors have been identified. Few receptors still remain orphans. What has emerged over the years is that most TNF ligands bind to one distinct receptor and some of the TNF ligands are able to bind to multiple TNF receptors, explaining to some extent the apparent disparity in the number of TNF receptors and ligands. Yet, in spite of some redundancy in TNF ligand/receptor interactions, it is clear that in vivo spatial, temporal, and indeed cell- and tissue-specific expression of both ligands and their receptors are important factors in determining the precise nature of cellular, physiological and pathological processes they control.

TNF superfamily has been the most highly investigated area of basic medical research for over two decades. These investigations have benefited from the enormous growth in our understanding of the principal functions of the immune system and the explosion in the knowledge involved in regulation of normal and pathological immune response. In addition, much has been learned about the molecular mechanisms of programmed cell death and the escape of tumor cells from apoptotic demise and from discovery of the key role played by TNF ligands in this process. As the functioning of these superfamily members is very complex, understanding TNF ligands and their receptor biology requires a mélange of research activities in many different disciplines including organ development, molecular biology, experimental pathology, and immunology. As a consequence of intensive studies in multiple areas over many years, much has been learned. A key role of members of this superfamily

in normal functioning of the immune system, autoimmunity, and other fundamental cellular process by which tumor cells develop has been established. Many novel mechanisms involving TNF superfamily members in the disease development process have been defined, and a unified concept and new perspectives have also emerged. For example, abrasions in the innate immune system, not always considered critical in autoimmunity, have come under increasing attention. Additionaly, TNF-directed and not antigen- directed therapy has emerged as the most impressive therapeutic advance in managing autoimmunity in humans. These findings provide a foundation for novel drug design efforts that are poised to utilize newly acquired knowledge. Several of these strategies have already materialized into successful therapeutics such as use of TNF for cancers and anti-TNFα antibodies and TNFR-Fc for autoimmune diseases, and many have advanced to human clinical trials, while many more are still being tested in preclinical settings.

As in other rapidly evolving fields, these advances are not necessarily congruent and are often difficult to organize into a cogent whole. The aim of *Therapeutic Targets of the TNF Superfamily* is to make readily available the major research important in the exploitation of this family for developing therapeutic strategies for human diseases, in a single volume. Under the auspices of Landes Bioscience, I have undertaken the task to concisely consolidate current knowledge of key TNF superfamily members focusing on both basic aspects and their clinical application. In this volume, a number of leading scientists in the field cover many aspects of biology of TNF superfamily members, ranging from the cloning and characterization of TNF ligands and their receptors, through the use of animal models to study their functions in vivo and their exploitation for human therapeutic use. Each chapter also includes relevant background information and provides useful bibliography for a more detailed analysis, making the study of TNF ligands/receptors accessible at all levels of expertise.

I would like to express my sincere thanks to all of my contributors for their excellent effort and undertaking this project with such enthusiasm, to Cynthia Conomos and Ronald G. Landes for commissioning me to edit this volume, and Megan Klein and the staff of Landes Bioscience for help with publication coordination. This volume presents the state-of-the art account on the role of TNF superfamily members in the pathogenesis and their use in current intervention of cancers and autoimmune disease. This text will be highly valuable for investigators to understand the disease processes regulated by TNF superfamily members and to develop effective therapeutics. A view into the future, inspired by the comprehensive work presented in this volume, predicts that researchers studying TNF superfamily members will continue to make rapid progress in identifying relevant components to the disease process and new therapeutic strategies to target many human diseases including cancers, autoimmune disease, and others.

Iqbal S. Grewal, PhD

ABOUT THE EDITOR...

IQBAL S. GREWAL, PhD. is well-known in the field of T-cell co-stimulation and autoimmunity and has extensively investigated several members of the TNF superfamily and molecules important for lymphocyte co-stimulation. His research has focused on the basic molecular and cellular processes to determine the biological roles of these molecules in normal physiology and immunity and their potential utility as agents or targets for the treatment of autoimmune diseases and cancers. His experience in discovering and developing innovative protein-based biotherapeutics in many disease areas has translated some of his findings into key drug candidates for the treatment of autoimmune disease and cancers.

Dr Grewal currently holds the position of Vice President of Preclinical Therapeutics at Seattle Genetics, Inc. in Bothell, Washington. He is responsible for preclinical translational research functions in support of the development of monoclonal antibodies and antibody-drug conjugates as therapeutics in the areas of autoimmunity and oncology. Before joining Seattle Genetics, Inc. Dr Grewal performed drug discovery research and preclinical development at Genentech in South San Francisco, California, where he identified and validated several novel molecules as therapeutic candidates in oncology and autoimmune disease. Prior to Genentech, Dr Grewal worked at Yale University School of Medicine. Before that, he held various research positions at the University of California, Los Angeles (UCLA). Dr Grewal has presented his work at both national and international meetings, as well as published over 100 scientific publications, 75 abstracts, 60 patent applications. He is a fellow of the Royal College of Pathologists, London and member of several distinguished societies. Dr Grewal holds a PhD in Immunology from UCLA and completed his post-doctoral fellowship at Howard Hughes Medical Institute at Yale University School of Medicine.

PARTICIPANTS

Bharat B. Aggarwal
Cytokine Research Laboratory
Department of Experimental Therapeutics
The University of Texas MD Anderson Cancer Center
Houston, Texas
USA

Robert Benschop
Lilly Research Laboratories
Eli Lilly and Co.
Indianapolis, Indiana
USA

Tamar Boursalian
Department of Preclinical Therapeutics
Seattle Genetics, Inc.
Bothell, Washington
USA

Michael Croft
Molecular Immunology
La Jolla Institute for Allergy and Immunology
La Jolla, California
USA

Martin Ehrenschwender
Department of Molecular Internal Medicine
Medical Clinic and Polyclinic II
University of Wuerzburg
Wuerzburg
Germany

Christina Falschlehner
Department of Immunology
Division of Medicine
Imperial College London
London
UK

Tom M. Ganten
Internal Medicine
University of Heidelberg
Heidelberg
Germany

Hans Peter Gerber
Department of Preclinical Therapeutics
Seattle Genetics, Inc.
Bothell, Washington
USA

Michael J. Gough
Robert W. Franz Cancer Center
Earle A. Chiles Research Institute
Providence Portland Medical Center
Portland, Oregon
USA

Iqbal S. Grewal
Department of Preclinical Therapeutics
Seattle Genetics, Inc.
Bothell, Washington
USA

Ronald Koschny
Internal Medicine
University of Heidelberg
Heidelberg
Germany

Ajaikumar B. Kunnumakkara
Cytokine Research Laboratory
Department of Experimental Therapeutics
The University of Texas MD Anderson Cancer Center
Houston, Texas
USA

Che-Leung Law
Department of Preclinical Therapeutics
Seattle Genetics, Inc.
Bothell, Washington
USA

Seung-Woo Lee
Molecular Immunology
La Jolla Institute for Allergy and Immunology
La Jolla, California
USA

Andreas Leibbrandt
Institute of Molecular Biotechnology of the Austrian Academy of Sciences
Vienna
Austria

Julie McEarchern
Department of Preclinical Therapeutics
Seattle Genetics, Inc.
Bothell, Washington
USA

Songqing Na
Lilly Research Laboratories
Eli Lilly and Co.
Indianapolis, Indiana
USA

Giuseppe Nocentini
Dipartimento di Medicina Clinica e Sperimentale
Sezione di Farmacologia Tossicologia e Chemioterapia
Università di Perugia; IBiT Foundation, Perugia
Polo Scientifico e Didattico di Terni
Perugia
Italy

Ezogelin Oflazoglu
Department of Preclinical Therapeutics
Seattle Genetics, Inc.
Bothell, Washington
USA

Josef M. Penninger
Institute of Molecular Biotechnology of the Austrian Academy of Sciences
Vienna
Austria

Carlo Riccardi
Dipartimento di Medicina Clinica e Sperimentale
Sezione di Farmacologia, Tossicologia e Chemioterapia
Università di Perugia; IBiT Foundation, Perugia
Polo Scientifico e Didattico di Terni
Perugia
Italy

Maureen C. Ryan
Seattle Genetics, Inc.
Bothell, Washington
USA

Uta Schaefer
Division of Apoptosis Regulation
German Cancer Research Center
Heidelberg
Germany

Gautam Sethi
Cytokine Research Laboratory
Department of Experimental Therapeutics
The University of Texas MD Anderson Cancer Center
Houston, Texas
USA

Bokyung Sung
Cytokine Research Laboratory
Department of Experimental Therapeutics
The University of Texas MD Anderson Cancer Center
Houston, Texas
USA

Harald Wajant
Department of Molecular Internal Medicine
Medical Clinic and Polyclinic II
University of Wuerzburg
Wuerzburg
Germany

Henning Walczak
Department of Immunology
Division of Medicine
Imperial College London
London
UK

Carl F. Ware
Division of Molecular Immunology
La Jolla Institute for Allergy and Immunology
La Jolla, California
USA

Tao Wei
Lilly Research Laboratories
Eli Lilly and Co.
Indianapolis, Indiana
USA

Andrew D. Weinberg
Robert W. Franz Cancer Center
Earle A. Chiles Research Institute
Providence Portland Medical Center
Portland, Oregon
USA

CONTENTS

Chapter 1

Overview of TNF Superfamily:
A Chest Full of Potential Therapeutic Targets

Iqbal S. Grewal*

Abstract

Since the discovery of tumor necrosis factor TNFα about 25 years ago, TNF superfamily has grown to a large family of related proteins consisting of over 20 members that signal through over 30 receptors. Members of this superfamily have wide tissue distribution and play important roles ranging from regulation of the normal biological processes such as immune responses, hematopoiesis and morphogenesis to their role in tumorigenesis, transplant rejection, septic shock, viral replication, bone resorption and autoimmunity. Thus, many approaches to harness the potency of TNF superfamily members to treat human diseases have been developed. Indeed, TNF and TNF agonistic molecules have been approved for human use in the United States and other countries. Many other TNF family members show promise for several therapeutic applications, including cancer, infectious disease, transplantation and autoimmunity. This chapter will give overview of TNF superfamily for exploitation for therapeutic use in humans.

Introduction

In middle of the nineteenth century, a surprising observation was made that in some cancer patients spontaneous regression of their tumors occurred if they were infected with bacterial infections.[1] This landmark discovery led to the idea of existence of a tumor necrotizing molecule and use of Coley's toxins (bacterial extracts) for the treatment of human cancers.[2] A century later, a factor from bacterial extracts, lipopolysaccharide (LPS), was isolated that was identified to be responsible for anti-tumor effects.[3] This effect of LPS on tumor regression was later shown to be due to induction of a factor in the serum. This factor was named as tumor-necrotizing factor[4] and later designated as tumor-necrosis factor (TNF).[5] Subsequently TNF was isolated[6] and its gene was cloned[7] and TNF became the prototype of a rapidly growing family of related proteins now called the TNF superfamily.

The TNF superfamily is now composed of over 20 TNF-related ligands all sharing many key structural features. A majority of these ligands are synthesized as type II transmembrane proteins. These ligands contain a relatively long extracellular domain and a short cytoplasmic region.[8] Their extracellular domains can be cleaved by specific metalloproteinases to generate a soluble molecule. In general, cleaved and noncleaved ligands are active as noncovalent homotrimers, although some members can also exist as heterotrimers. Both membrane-bound and secreted ligands are expressed by a variety of normal and malignant cell types.[9] Since most of TNF-superfamily members are expressed as transmembrane cell surface proteins, it is believed they are acting at a local level. Key members of this family include APRIL, BAFF, 4-1BBL, CD30L, CD40L, CD70, CD95L, OX40L, LTα, LTβ, RANKL, NGF, TNFα and TRAIL (Fig. 1). More than 30 receptors for the TNF ligands belonging to the TNF receptor (TNFR) superfamily have been identified in

*Iqbal S. Grewal—Seattle Genetics, Inc., 21823 30th Drive SE, Bothell, WA 98021, USA. Email: igrewal@seagen.com

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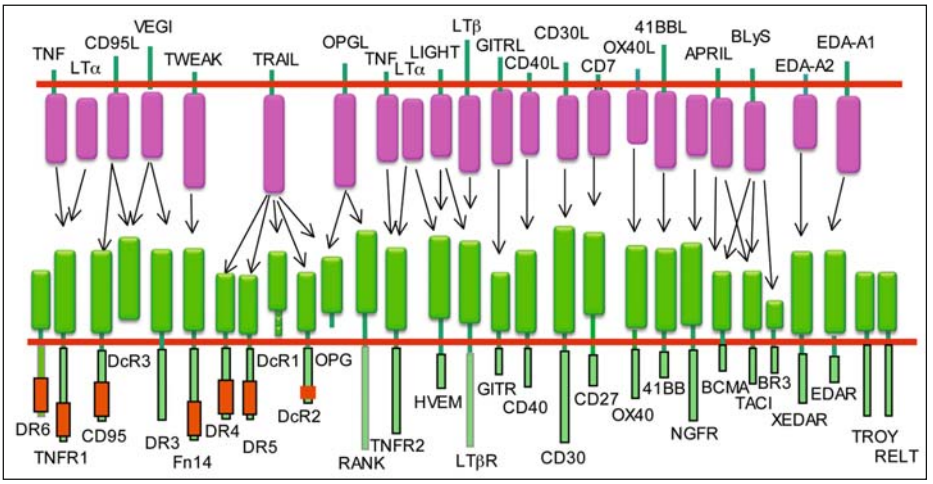

Figure 1. TNF superfamily ligands and their known receptors. Ligands and their receptors are shown in a diagramatic form. Many ligands bind to more than one receptor as indicated by arrows. Ligands for DR6, TROY and RELT have not yet been discovered. Red boxes shown in the cytoplasmic part of the receptors indicate presence of a death domain. A color version of this image is available at www.eurekah.com

humans and in mice. These receptors are type I membrane proteins characterized by the presence of a distinctive cystein-rich domain in their extra-cellular portion.[9] Most of TNF ligands bind to a single receptor, but few of them bind to more than one receptor. For example, TRAIL is known to bind five receptors (DR4, DR5, DcR1, DcR2 and OPG). TNF receptors exert their cellular responses through signaling sequences in their cytoplasmic regions.

Based upon these cytoplasmic sequences and signaling properties, the TNF receptors can be classified into three major groups.[10] The first group includes receptors that contain a death domain (DD) in their cytoplasmic tail. These receptors include CD95, TNFR1, DR3, DR4, DR5 and DR6. Binding of TNF superfamily ligands to their DD containing receptors causes complex signaling through adaptor proteins, such as tumor necrosis factor receptor—associated death domain (TRADD), resulting in activation of the caspase cascade and apoptosis of the cell.[11] The second groups of receptors contain one or more TNF receptor-associated factors (TRAF) interacting motifs (TIM) in their cytoplasmic tails. This group includes TNFR2, CD40, CD30, CD27, LT-βR, OX40, 4-1BB, BAFFR, BCMA, TACI, RANK, NGFR, HVEM, GITR, TROY, EDAR, XEDAR, RELT and Fn14.[12] Ligand binding to TIM containing TNF receptors induces recruitment of TRAF family members and activation of cellular signaling pathways including activation of a nuclear factor-κB (NF-κB), Jun N-terminal kinase (JNK), p38, extracellular signal regulated kinase (ERK) and phosphoinisitide-3 kinase.[12] The third group of TNF receptor family members does not contain functional intracellular signaling domains or motifs. These receptors include DcR1, DcR2, DcR3 and OPG. Although this group of receptors lacks the ability to provide intracellular signaling, they can effectively act as decoys to compete for ligand binding and block the signaling through other two groups of receptors.[13]

Signaling events induced by TNFR superfamily members regulate a very broad array of developmental processes and play pivotal roles in numerous biological events in mammals including induction of apoptosis, survival, differentiation and proliferation of cells. The majority of TNF superfamily ligands are predominantly expressed on cells involved in the immune system including B-cells, T-cells, NK cells, monocytes and dendritic cells. In contrast, TNF receptors are expressed by a wide variety of cells that have both hematopoetic and nonhematopoeitic

origins. Thus, numerous activities are assigned to TNF superfamily members, in particular, their profound role in regulation of both normal and pathogenic immune responses.

The strong role played by TNF superfamily members in normal development is illustrated by the identification of disease causing mutations in both ligands and receptors. Humans with mutations in TNFR1, CD95, CD95L, RANK, EDA and CD40L manifest profound abnormalities. For example, mutations in TNFR1 are linked to periodic fever syndromes called TNFR1-associated periodic syndromes, which is manifested as unexplained episodes of fever and severe localized inflammation.[14] Individuals who have mutations in CD95 and CD95L manifest autoimmune lymphoproliferative syndrome (ALPS),[15] a phenotype that is similar to those of lpr and gld mice, which also have mutations in CD95 and CD95L respectively.[16] Because of lack of CD95 signals, ALPS patients have defective lymphocyte apoptosis and have an increased risk of developing T- and B-cell lymphomas.[15] Humans with mutations in CD40L gene, an X-linked hyper-IgM syndrome, have severe defects in immunoglobulin isotype switching mechanisms. This is illustrated by the accumulation of large amounts of IgM in the serum of patients with the CD40L gene mutation and their susceptibility to opportunistic infections.[17] Two mutations in RANK gene are linked to familial expansile osteolysis, a rare autosomal dominant bone disorder which is characterized by focal areas of increased bone remodeling. In this disease, an increased activity of osteoblasts and osteoclasts causes the osteolytic lesions to develop in the long bones during early adulthood. Both of these mutations have been linked to an increase in RANK-mediated NF-κB signaling in vitro, consistent with observed phenotype in vivo in humans.[18] Mutations in EDA results in hypohydrotic ectodermal dysplasia, both in humans and mice, which is characterized by the loss of hair, sweat glands and teeth.[19] A similar phenotype is also seen with mutations in receptor for EDA. EDA regulates the initiation and morphogenesis of hair and teeth by activating NF-κB in the ectoderm. Additionally, mutations in death receptors such as CD95 and DR4 have been linked to various carcinomas and lymphomas.[22,21] A critical in vivo role for BR3 (receptor for BAFF) in B-cell ontogeny is also illustrated by the finding of severe deficiency in B-cell development in mice with mutations in *br3* gene.[22]

Therapeutic Potential of TNF Superfamily for Anticancer Treatment

As mentioned earlier, a group of TNF superfamily receptors are death receptors and have the unique ability to transmit an intracellular death signal and TNF ligands such as TNF, CD95L and TRAIL are capable of inducing apoptosis in tumor cells. Because TNF receptors are expressed on many tumor cells, TNF was exploited for its antitumor effects. Its use as an anti-cancer agent, however, is limited because of its systemic toxicity.[23] Many approaches are being considered to improve its toxicity profile. For example, inhibition of matrix metalloproteinases has been documented to reduce the toxicity induced by TNF.[24] Furthermore, TNF has been successfully used to treat limb soft-tissue sarcomas and other large tumors when administered locally by isolated limb perfusion to avoid its systemic effects.[25] Similar to the use of TNF, in vivo use of CD95L is also limited by its lethal hepatotoxicity resulting from massive hepatocyte apoptosis. This led to testing of other TNF ligands that may selectively affect tumor survival in humans without significant toxicity. When TRAIL was investigated in this context, it was discovered that TRAIL can specifically kill tumor cells without harming normal cells, suggesting it could be used for the treatment of cancer. This is supported by a myriad of animal studies investigating the anticancer potential of TRAIL where very promising results have been seen.[26,27] In addition to TRAIL, agonistic antibodies specific for its receptors DR4[28] and DR5[29] are also being explored for their antitumor effects and are now in clinical testing. Since a number of other TNF family members such as, CD30, CD40, CD70, BR3, BCMA are also expressed on tumor cells, antibody based therapies are being developed to target these molecules and are currently undergoing clinical trials. In addition, some of these molecules are also being exploited as targets for antibody-drug conjugates (CD30 and CD70) and for radioimmunotherapy (BLyS receptors TACI and BR3).[30]

As TNF superfamily members play a profound role in the activation of immune response, therapeutic approaches to target TNF superfamily members to boost anti-tumor host responses

have also been developed. Use of agonistic molecules to target TNF receptors for some members of the family are being tested in the clinic. These include, agonistic anti-CD40 antibodies, soluble CD40L and OX40L.[31,32] In cases, such as CD40, where the target is also expressed on tumor cells, rationally designed antibody-based therapies combining the induction of tumor cell apoptosis and activation of tumor-specific adaptive immunity can be considered. This will enable promotion of antitumor host immune response and could help to eradicate large established and heterogeneous tumors without requiring expression of tumor-specific antigens on all tumor cells.

Therapeutic Potential of TNF Superfamily for Autoimmune and Inflammatory Disease

It is clear now that the immune system is regulated not only by cell proliferation and differentiation but also by apoptosis and TNF superfamily members control and orchestrate the immune response at multiple levels.[33] Thus, TNF superfamily members have been implicated in both innate and adaptive immune responses such as defense against pathogens, inflammatory response and autoimmunity, as well as the normal development of the immune system.[33,34] TNF superfamily members such as TNF, LTα, LTβ and RANKL provide crucial role in the development of secondary lymphoid organs. CD95 is important for the regulation of immune system for both the development of T-cells in thymus and for the induction of apoptosis of activated T-cells important for the downregulation the immune response.[35] TNF superfamily members, such as TNF, CD95L and TRAIL, also contribute to the cytotoxic effector cell response in the recognition and elimination of virus-infected cells. Some members of the family are also important for the integrity of the host tissues. For example, the eye is maintained as an immune-privileged site by the expression of CD95L by the corneal cells. CD95L kills the inflammatory cells and protects the eye from inflammatory attack.[36] Similarly, TRAIL has also been shown to have an important role in regulation of lymphocyte functions in the periphery by inhibiting cell-cycle progression by T-cells.[37]

Since most of the TNF ligands are expressed by the cells of the immune systems, their crucial role in regulation and proliferation of B-cells, T-cells and monocytes as well as in homeostasis has been well documented.[38] Thus, importance of CD95L in peripheral T-cell homeostasis and crucial role of 4-1BBL, CD27, CD30L, OX40 and CD40L in costimulation T-cells has been well described.[34] As CD30, CD40 and receptors for TNF, LT, APRIL, BAFF and CD70 are expressed on B-cells these family members are implicated in the control of maturation and survival of B-cells.[11,39] Many TNF superfamily members are also important for the activation and development of effector cells such as CD40L for B-cells; 4-1BBL, OX40L and CD70 for T-cells; and CD40 and RANKL for dendritic cells.[38] In addition, several TNF ligands such as BAFF, CD30L, CD40L, OX40L, 4-1BBL and CD70 have been implicated in the development of autoimmunity.[38] A potential role of TRAIL and other members of the TNF superfamily in organ transplantation has also been documented.[40] A profound role of TNF superfamily members such as CD40L and TNFα has been described for protective Th1-type host response against infection with bacteria. TNFα has been also implicated in LPS-mediated septic shock. In addition to their profound role in autoimmune and inflammatory response, members of the TNF superfamily have also been implicated in chronic heart failure, bone resorption, AIDS, Alzheimer's disease, transplant rejection and atherosolerosis.[9]

Accordingly, approaches to target many of TNF superfamily receptors and ligands for treatment of autoimmunity and other inflammatory diseases are being exploited. Indeed, a number of biologic TNF blocking therapies including humanized monoclonal antibodies (e.g., infliximab or adalimumab) or recombinant fusion proteins of IgG and soluble receptors (e.g., etanercept) have been developed and are being used in humans now to inhibit the inflammation associated with Crohn's disease and rheumatoid arthritis.[41] TNF receptor fusion proteins are also in development for heart disease. In addition number of other therapies targeting TNF superfamily including TACI-FC, BR3-Fc, anti-BLyS, anti-CD70, anti-OX40L and anti-CD40L are currently in clinical testing.

Challenges for Targeting TNF Superfamily Members

Although soluble TNF and anti-TNF agents have been approved for human therapies and are being successfully used in human patients, there are number of toxicities associated with these therapies. At the time of the discovery of TNF, the main role attributed to this cytokine was in tumor killing, however, it is now evident that TNF can also play a much broader role in tumorigenesis. The molecular mechanism of action of TNF is mediated through the activation of NF-κB, which is known to regulate various molecules involved in invasion and metastasis of the tumors. In addition, TNF use in humans leads to direct toxicity of the liver resulting in damage of hepatocytes. Similar to the use of TNF, CD95L use also results in hepatotoxicity, suggesting a similar mechanism of action of CD95L. The continued examination of signal transduction of TNF superfamily members is needed to develop approaches for tissue specific interventions, which could allow targeted therapies to have fewer side effects.

Three different antibodies against CD40L were tested in clinical trials for potential therapies for autoimmune and inflammatory disease. However, thromboembolic complications were associated with all three antibodies, which led to a complete stop in clinical testing of these antibodies.[42] Mechanism underlying anti-CD40L antibody induced thromboembolism remains to be elucidated. This issue is discussed in detail in reference 31, where alternative approaches to target CD40L are being considered.

Increased risk of development of tuberculosis and lymphoma is also seen with anti-TNF therapies.[43] This is consistent with the important role of TNF in regulation of many cellular processes and in activation of macrophages which are key players in killing *Mycobacterium tuberculosis*. Several of the harmful effects of TNF and its superfamily members are thought to be mediated through the activation of NF-κB. As all TNF superfamily members have potential to activate NF-κB and induce large numbers of genes, it is not surprising that their products are implicated in a wide variety of toxicities. Thus the beneficial role of therapies targeting TNF family members and their potential immunosuppressive or toxic effects must be critically examined in both animal studies and in human trials.

Summary and Conclusions

It is clear that over 20 members of the TNF superfamily and their receptors have been identified and these molecules have a wide-ranging role in many cellular and physiological functions. The key mechanism of the action of these molecules includes activation of NF-κB, JNK, p38 MAPK and ERK1/ERK2. Signals stemming from TNF-superfamily members promote multiple cellular events including apoptosis, proliferation, survival and differentiation. These molecules are also shown to mediate hematopoiesis, immune surveillance, tumor growth and protection from infection. In addition TNF superfamily members are also implicated in inflammatory immune response, autoimmunity, septic shock and osteoporosis. Based on the profound role the TNF superfamily plays in multiple cellular events it is no surprise that many approaches to target this family for therapeutic use are being developed. Indeed, TNF and its inhibitors have been approved as therapeutics. These include TNF for human use in the treatment of sarcomas and melanomas,[25,44] anti-TNF antibodies for rheumatoid arthritis and Crohn's disease,[45] Anti-TNF therapy using soluble TNFR2 for rheumatoid arthritis.[41] A number of new molecules targeting TNF superfamily are also being exploited for the treatment of cancers and various autoimmune diseases. How several members of the TNF superfamily are exploited for the therapeutic use is discussed in detail in the following chapters.

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Chapter 2

Therapeutic Interventions Targeting CD40L (CD154) and CD40:
The Opportunities and Challenges

Che-Leung Law* and Iqbal S. Grewal

Abstract

CD40 was originally identified as a receptor on B-cells that delivers contact-dependent T helper signals to B-cells through interaction with CD40 ligand (CD40L, CD154). The pivotal role played by CD40-CD40L interaction is illustrated by the defects in B-lineage cell development and the altered structures of secondary lymphoid tissues in patients and engineered mice deficient in CD40 or CD40L. CD40 signaling also provides critical functions in stimulating antigen presentation, priming of helper and cytotoxic T-cells and a variety of inflammatory reactions. As such, dysregulations in the CD40-CD40L costimulation pathway are prominently featured in human diseases ranging from inflammatory conditions to systemic autoimmunity and tissue-specific autoimmune diseases. Moreover, studies in CD40-expressing cancers have provided convincing evidence that the CD40-CD40L pathway regulates survival of neoplastic cells as well as presentation of tumor-associated antigens to the immune system. Extensive research has been devoted to explore CD40 and CD40L as drug targets. A number of anti-CD40L and anti-CD40 antibodies with diverse biological effects are in clinical development for treatment of cancer and autoimmune diseases. This chapter reviews the role of CD40-CD40L costimulation in disease pathogenesis, the characteristics of therapeutic agents targeting this pathway and status of their clinical development.

Introduction

CD40 (TNFRSF5), a member of the tumor necrosis factor (TNF) receptor superfamily, is a signaling cell surface receptor. Sequence motifs involved in CD40-mediated signal transduction have been identified in the CD40 cytoplasmic tail that interact with the TNFR-associated factors (TRAFs) to trigger downstream signal cascades that in turn modulate the transcriptional activities of a variety of survival and growth-related genes.[1-3] CD40 is expressed on B-cells at multiple stages of differentiation, monocytes, macrophages, platelets, follicular dendritic cells, dendritic cells (DCs), eosinophils and activated $CD8^+$ T-cells.[4-6] In non-hematopoietic tissues, CD40 is expressed on thymus and kidney epithelial cells, keratinocytes, synovial membrane and dermal fibroblasts and activated endothelium.[7-9] The endogenous ligand for CD40 is CD40L (CD154, TNFSF5).[4,5,7,10] Expression of CD40L on T-cells is tightly regulated; it is only transiently expressed on activated $CD4^+$, $CD8^+$ and γδ T-cells.[11] Besides activated T-cells, CD40L is expressed by monocytes, activated B-cells, epithelial and vascular endothelial cells, smooth muscle cells and DCs. The functional relevance of CD40L expression on these cell types remains to be

*Corresponding Author: Che-Leung Law—Department of Preclinical Therapeutics, Seattle Genetics Inc., 21823 30th Drive SE, Bothell, Washington 98021, USA.
Email: claw@seagen.com

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fully understood.[5] However, expression of CD40L on activated platelets may participate in the pathogenesis of thrombotic diseases.[6,12,13]

The best characterized function of the CD40-CD40L interaction is in contact-dependent reciprocal interaction between antigen-presenting cells (APCs) and T-cells.[10,14] On resting B-cells, binding of CD40L to CD40 promotes B-cell survival and activation, drives rapid expansion of antigen-activated B-cells and facilitates plasma cells and memory B-cell differentiation. CD40 signaling is required for germinal center formation, immunoglobulin (Ig) gene somatic hypermutation, affinity maturation and isotype switching. The physiological importance of CD40 signaling is illustrated by patients suffering from the X-linked hyper-IgM (XHIGM) syndrome.[15] In this primary immunodeficiency, mutations in the CD40L locus abolish functional CD40-CD40L interaction. Disease manifestations include over-representation of circulating IgM and the inability to produce IgG, IgA and IgE. Consequently, patients have profoundly suppressed secondary humoral immune responses, increased susceptibility to recurrent infections and a higher frequency of developing cancer.[16-20]

Genetic deletion of either the *Cd40* or *Cd40l* locus in mice reproduces the major defects seen in XHIGM patients. The most prominent defect seen in the $CD40^{-/-}$ mice is the failure to form germinal centers. Thymus-independent IgG and IgM responses remain relatively normal in these mice, but antibody responses to thymus-dependent antigens and antibody class-switching are suppressed.[21,22] Similar to the $CD40^{-/-}$ mice, the primary defects in $CD40L^{-/-}$ mice also reside in the B-cell compartment.[23-27] Ineffective priming of T-cells to the immunizing protein antigen appears to be the major culprit behind the reduced humoral response in the $CD40L^{-/-}$ mice.[28]

In the T-cell compartment, functional differentiation of CD4 and CD8 cells have different requirements for CD40-CD40L costimulation compared to B-lineage cells. Helper Th-cell-mediated functions including local inflammatory reactions to lymphocytic choriomeningitis virus (LCMV) infection and ability to clear secondary viral infection are minimally affected in $CD40^{-/-}$ or $CD40L^{-/-}$ mice.[29] Likewise, $CD40L^{-/-}$ mice can mount potent, primary virus-specific CD8 T-cell responses against LCMV, Pichinde virus and vesicular stomatitis virus.[27,29-31] In contrast, the memory Cytolic T-Lymphocyte (CTL) response in $CD40L^{-/-}$ mice is much less efficient than in wild-type mice even though memory CTL activity is detectable in $CD40L^{-/-}$ mice.[27,32] Inadequate Th cell priming in these $CD40L^{-/-}$ mice is believed to be the main reason behind their diminished memory CTL responses.[27,31,32] Functional T-cell-macrophage interaction in $CD40L^{-/-}$ mice is also hampered, resulting in altered macrophage-mediated inflammatory responses, heightened susceptibility to infection by *Leishmania amazonensis* and failure to generate a protective secondary immune response against the parasite.[33]

CD40 signaling to DCs is probably the most important requirement in T-cell priming. CD40 ligation on DCs up-regulates MHC class II antigens, the costimulatory molecules CD80, CD86 and CD70 and adhesion molecules including CD54, thereby promoting antigen presentation, inducing DC maturation and enhancing their costimulatory activity.[5,10] Mature DCs stimulate activated T-cells to increase IL-2 production that facilitates Th and CTL expansion.[28,34,35] CD40-stimulated DCs also secrete IL-12, TNFα, IL-8 and MIP-1α that favor Th1 cell differentiation and promote Th cell migration to sites of inflammation.[36-38] In addition, CD40-activated DC cells presenting antigens in the context of MHC class I molecules are potent stimulators of CTL precursors, a process known as cross-priming, which is critical for cell-mediated immunity against viral infection and transformed cells expressing tumor-associated antigens.[39,40] The importance of CD40 in CTL cross-priming is confirmed by the observation that administration of agonistic monoclonal antibodies (mAbs) against CD40 is sufficient to substitute the need of Th-cells for the generation of robust CTL responses.[41]

In the reticuloendothelial system, CD40 ligation provides a pro-inflammatory signal. Triggering CD40 up-regulates the adhesion molecules CD54, E-selectin and VCAM-1 on monocytes, fibroblasts, keratinocytes, smooth muscle cells and activated endothelial cells.[8,10,36,42] At the same time, the pro-inflammatory cytokines IL-1, IL-6, IL-12, IFNγ and TNFα are secreted by these