Am. J. Hum. Genet. 65:294–301, 1999

IMMUNOGENETICS '99
Unlinking Tumor Necrosis Factor Biology from the Major Histocompatibility Complex: Lessons from Human Genetics and Animal Models

Sigrid R. Ruuls and Jonathon D. Sedgwick

DNAX Research Institute, Palo Alto, CA

Introduction

Tumor necrosis factor (TNF, originally cachectin or TNF-α) is the prototype member of a large family of proteins with diverse functions including induction of apoptosis and regulation of lymphocyte proliferation. These ligands bind and signal through members of the TNF-receptor (TNFR) family of cell-surface receptors (Bazzoni and Beutler 1996). TNF itself can exhibit an extraordinarily broad spectrum of biological effects. In T cell–dependent immune responses, for example, TNF can, in principal, play critical roles in the induction, perpetuation, and resolution of the host response (fig. 1). It is not surprising, therefore, that, when TNF is absent or its activity is blocked, wide-ranging physiological and immunologic processes fail. These range from increased susceptibility to intracellular bacterial infection to the loss of normal lymphoid tissue microarchitecture.

Dysregulation and, in particular, overproduction of TNF has been implicated in a variety of human diseases including sepsis, cerebral malaria, and autoimmune diseases such as multiple sclerosis, rheumatoid arthritis (RA), systemic lupus erythematosus, and Crohn disease, as well as cancer (reviewed in Zhang and Tracey 1998). Susceptibility to many of these diseases is thought to have a genetic basis, and the *TNF* gene is considered a candidate-predisposing gene. However, unraveling the importance of genetic variation in the *TNF* locus to disease susceptibility or severity is complicated by its location within the major histocompatibility complex (MHC), a highly polymorphic region that encodes numerous genes involved in immunologic responses (reviewed in Gruen and Weissman 1997). In this report, we review studies that analyze the contribution of TNF and related genes to susceptibility to human disease. Furthermore, we discuss how the presence of the TNF locus within the MHC may potentially complicate the interpretation of studies in animal models in which the TNF locus is experimentally manipulated.

Received June 4, 1999; accepted for publication June 9, 1999; electronically published July 7, 1999.

Address for correspondence and reprints: Dr. Jonathon D. Sedgwick, DNAX Research Institute, Immunobiology Department, 901 California Avenue, Palo Alto, CA 94304-1104. E-mail: jon.sedgwick@dnax.org

This article represents the opinion of the authors and has not been peer reviewed.

© 1999 by The American Society of Human Genetics. All rights reserved.

0002-9297/99/6502-0004$02.00

Genomic Localization and Molecular Forms of TNF and Related Molecules

The human MHC gene cluster, localized on chromosome 6 (chromosome 17 in the mouse), is divided into four regions (fig. 2). Class I HLA genes are found at the telomeric end of the cluster, with class II genes at the centromeric end. Two other extended loci are the class III region, which encodes several components of the complement system, and the more recently demarcated class IV region. The *TNF* gene, along with two related genes, encoding lymphotoxin-α (*LT*α, previously known as TNFβ) and *LT*β (Gruen and Weissman 1997), occur within the MHC class IV cluster.

TNF can be produced by a wide variety of cells, but the activated macrophage constitutes the most abundant source of this factor (Vassalli 1992). TNF is first produced as a cell-membrane–bound, 26-kD molecule that is cleaved from the cell surface by a metalloproteinase disintegrin TACE (TNF-α converting enzyme) to generate a secreted 17-kD form of the TNF molecule (fig. 3). Membrane as well as secreted forms of TNF trimerize and interact as homotrimers with two TNF receptors—TNF-R1 (human p60, mouse p55) and TNF-R2 (human p80, mouse p75). Both receptors are initially integral membrane proteins but can be cleaved from the cell surface, to become bioactive soluble molecules (reviewed in Bazzoni and Beutler 1996).

LTα expression is much more restricted than that of TNF and is predominantly, if not exclusively, found in T lymphocytes, B cells, and NK cells (Beutler 1992). LTα is distinct from TNF in having no transmembrane region, although, like TNF, it can form a soluble homo-

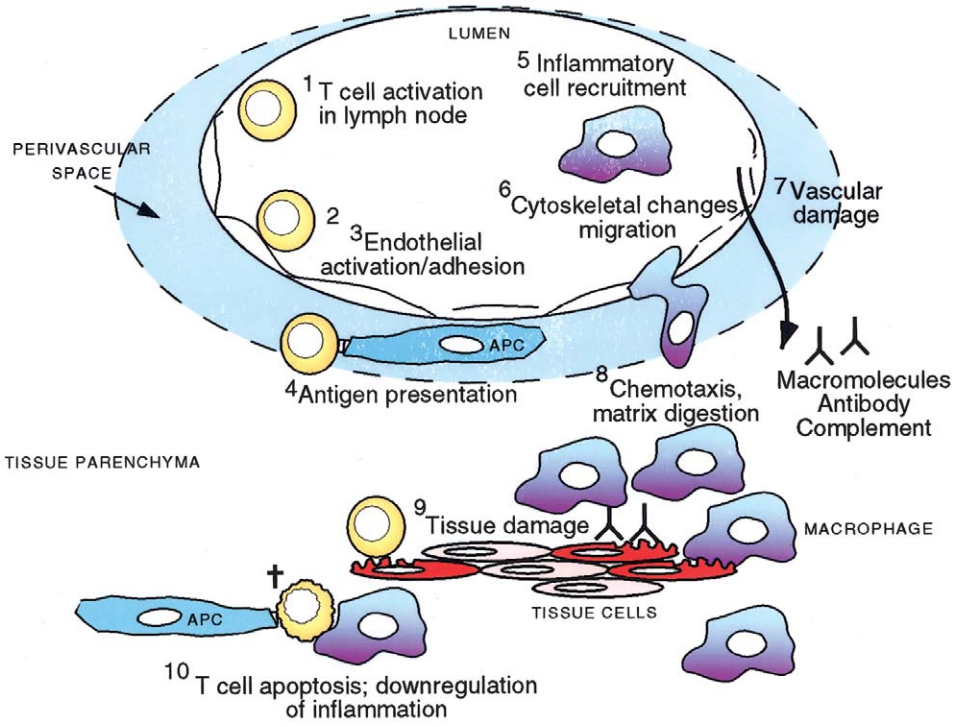

Figure 1 TNF actions in inflammation from genesis to resolution. TNF may contribute to each step of inflammation by any of the following mechanisms. Step 1, Organization of secondary lymphoid organs and dendritic cell maturation. Steps 2 and 3, Regulation of adhesion molecule expression. Step 4, Up-regulation of MHC expression in synergy with other cytokines. Step 5, Induction of leukocyte activation and adhesion. Step 6, Chemoattractant production by endothelial cells, facilitating leukocyte transmigration. Step 7, Rearrangement of endothelial cell cytoskeleton, causing vascular leakage. Step 8, Induction of chemokine secretion by stromal cells and inflammatory leukocytes; protease release by leukocytes. Step 9, Direct cytotoxicity, induction of secondary inflammatory mediators and cytotoxic molecules. Step 10, Induction of down-regulatory molecules, direct effect of T cell apoptosis. APC = antigen-presenting cell.

trimer (LTα3) that interacts with the same two TNF receptors (fig. 3; Bazzoni and Beutler 1996). The physiological significance of this interaction, at least in mice, has been questioned (Mackay et al. 1997), although there is evidence that interactions between LTα3 and TNF-R1 may promote formation of lymphoid aggregates in regions of chronic inflammation (Sacca et al. 1998). LTα forms a heterotrimer with LTβ—an integral membrane protein that acts as a membrane anchor—to generate LTα1β2 (see fig. 3), which interacts with a distinct receptor, the LTβ receptor (LTβR, Bazzoni and Beutler 1996). Because LTβ is expressed more widely than LTα, the formation of this heterotrimer may be controlled by the rate-limiting production of LTα.

Polymorphisms in the TNF Locus: Implications for Production and Consequences for Disease

A number of MHC class I and class II alleles have been positively identified as predisposing to various diseases, most of them autoimmune in nature (Theofilopoulos 1995). However, in other cases, initial low-resolution mapping of disease susceptibility to the MHC region has, upon closer examination, been shown to be independent of class I and class II genes. Other candidate genes that encode proteins involved in inflammation, stress responses, or host defense are located in the closely linked MHC class III and class IV region (Gruen and Weissman 1997). A number of studies have focused on the TNF locus as one of these candidates.

In rodents there is evidence that polymorphisms in an AU-rich element within the 3′ untranslated region of the TNF mRNA may underlie differences in inducible TNF production across a range of inbred mouse strains. To test this possibility, Kollias's group prepared gene-targeted mice that lack this element, and they found that TNF mRNA is abnormally stable in these animals and that the protein is produced by cells such as synovial fibroblasts, wherein TNF production is normally repressed. Mice with this mutation spontaneously developed an erosive arthritis (Kontoyiannis et al. 1999).

In humans, the significance of polymorphisms in *TNF* is much less clear. Healthy individuals exhibit a considerable variation in their levels of mitogen-induced TNF

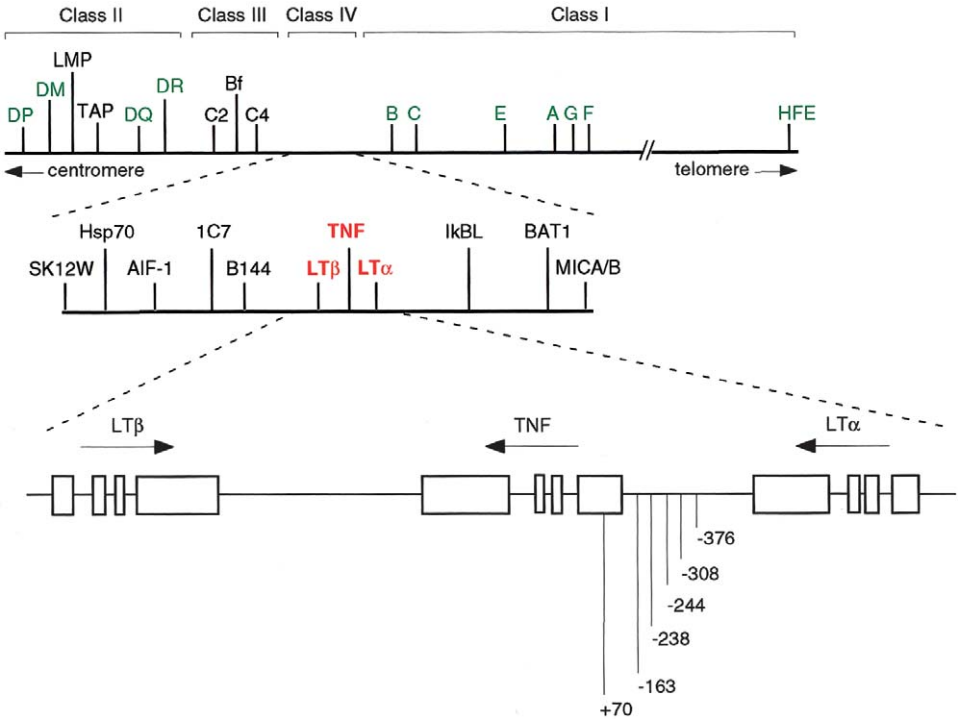

Figure 2 The human MHC region and the TNF-related genes. In this physical map of the MHC region, classic HLA class I and class II genes are depicted in green. The MHC class IV region is shown in greater detail on the middle line. In addition to TNF, LTα, and LTβ, class IV genes with possible functions in immunologic responses include SK12W (antiviral activity), heat shock protein 70 (Hsp70, putative role in autoimmune inflammation), allograft inflammatory factor-1 (AIF-1, allograft rejection), 1C7 (member of the Ig superfamily, expressed by monocytes, putative role in macrophage activation), B144 (or leukocyte-specific transcript-1, LST-1, involved in macrophage activation), IκB-like (IκBL, negative regulation of NFκB), HLA-B–associated transcript-1 (BAT1, RNA helicase involved in B144 splicing) and MHC class I–related genes A and B (MIC-A and MIC-B, heat-shock induced) (Gruen and Weissman 1997). The bottom line shows the genomic organization of the *LTβ, TNF,* and *LTα* genes. Open boxes represent exons. Arrows indicate transcriptional direction. Polymorphisms involving G→A transitions at positions −376, −308, −244, −238, and −163, and a C insertion at position +70 of TNF are shown.

production. TNF responses have been associated with certain HLA alleles: HLA-DR3 and -DR4 correlate with the "high-producing" phenotype for TNF, whereas HLA-DR2 occurs with the "low-producing" phenotype (Jacob et al. 1990). An alternative explanation for the association with class II alleles is that the differences reflect polymorphisms in closely linked gene(s), including the *TNF* gene itself. To date, nine polymorphisms and five microsatellites in the TNF locus have been characterized. Six of these polymorphisms are located in the *TNF* promotor region (fig. 2), and might, therefore, influence the transcription rate of this gene. Indeed, as reviewed in Wilson et al. (1995), TNF synthesis levels in peripheral blood may correlate with some of these polymorphisms. One well-studied polymorphism, which carries a G→A transition at position −308, is strongly associated with the high-producer HLA-DR3 allele and is implicated in the risk of death from cerebral malaria (Zhang and Tracey 1998). Wilson et al. (1997) raised considerable interest with their report that the −308A allele is transcribed in vitro at seven times the rate of

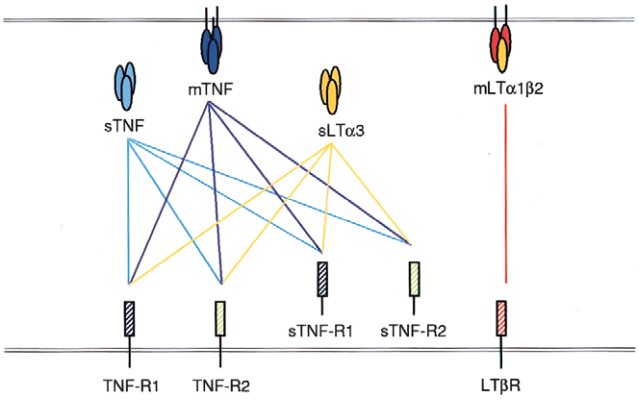

Figure 3 The interaction of TNF and LT proteins with their receptors. Receptors recognize trimeric forms of the TNF and LT ligands. There is speculation regarding the extent of oligomerization of receptors upon ligand binding (Bazzoni and Beutler 1996). s = secreted; m = membrane-associated.

the −308G allele, but this result has not been confirmed (Brinkman et al. 1996). Stuber et al. (1996) showed that the −308A allele does not affect the outcome of sepsis, as might have been supposed, given that high TNF production during sepsis confers a high risk of fatal shock.

A complicating factor in the search for disease-associated genes in the human population is that many diseases studied are very heterogeneous clinically and that certain genetic factors may not associate with the absence or presence of a disease but with its severity or course. For example, *TNF* polymorphisms have been shown to correlate to clinical outcome of RA (Brinkman et al. 1997). In a large group of RA patients, the *TNF* −238GA genotype was associated with the absence of erosive disease compared with the more typical −238GG genotype. Because similar frequencies of the *TNF* −238GA genotype are observed in RA patients and healthy controls, the effect of this polymorphism appears to be limited to disease modification, rather than to predisposition. Despite extensive research efforts, no human *TNF* allelic forms have been identified that result in substantial alterations in TNF expression or regulation, and no definitive disease-related *TNF* polymorphism has been characterized.

Even if a strong correlation between TNF levels and disease susceptibility or severity can be demonstrated, it would not necessarily follow that the defect lies in the *TNF* gene itself. Any gene involved in regulating the expression or activity of TNF may be the culprit. For example, soluble TNF receptors provide a further level of regulation, controlling the availability of bioactive TNF levels by binding and neutralizing secreted or membrane-bound TNF. Indeed, in mice deficient in TNF-R1 or TNF-R2, levels of inducible TNF are elevated substantially (Peschon et al. 1998). Possibly related to this effect is a recent study showing that mutations in the extracellular domain of human TNF-R1 are linked to dominantly inherited autoinflammatory syndromes (McDermott et al. 1999). These TNF-R1 mutations lead to decreased receptor shedding and a consequent increase in cell-surface expression of TNF-R1. How this produced the clinical syndromes remains unclear, but they may have occurred because (1) mutations in TNF-R1 result in increased levels of circulating TNF or (2) an increase in the stability of membrane-bound receptor results in some form of excessive overstimulation.

Animal Models for Human Disease: The Application and Limitations of Gene Targeting

The close genetic linkage between the TNF locus and other molecules within the MHC, many of which are plausible candidate genes in immunologic disorders, undoubtedly complicates the study of these human diseases. Less widely appreciated is the fact that the synteny of these genes is conserved in other model organisms, and there exists a similar challenge to analyses of these disease processes in genetically engineered mice. Inbred mouse strains differ substantially and reproducibly in their responses to different kinds of pathogens or antigens, and the relevance of HLA class I and class II genes to these phenotypes is a matter of considerable interest.

Gene targeting, which allows both knock-out and knock-in genotypes to be constructed, would appear to provide a useful tool to distinguish the effects of these polymorphisms from those that occur in *TNF* or other MHC class IV genes. However, gene targeting is usually performed using embryonic stem (ES) cells derived from the 129 mouse strain (Galli-Taliadoros et al. 1995), and the targeted mutation is routinely introduced into the genetic background of interest by means of multiple rounds of backcrossing. In common practice, experiments of this kind are generally reported after relatively few rounds of backcrossing, so there is often a considerable contribution of the 129 strain to the genotypes of the animals studied (Gerlai 1996). Moreover, with respect to mutations introduced into genes within the MHC, such as TNF and LTα, even if the standard 12 generations of backcrossing are performed (so that the animals with the targeted allele carry <0.5% of the 129 genotype), the mutation of interest will still most likely exist on an MHC background characteristic of the 129 strain. The small genetic distances within the MHC cluster introduce linkage disequilibrium among these genes. Worse still is the (usually sound) practice of using nontargeted littermates as controls in these experiments. Because of linkage disequilibrium, the targeted mutation will not be the only difference between the experimental and the control group.

The most satisfactory solution to this problem is to create targeted mutations in ES cells derived from the exact strain in which studies are to be performed. In this way, all backcrossing can be avoided. In our studies of experimental autoimmune encephalomyelitis (EAE)—an animal model for the human disease multiple sclerosis—we used ES cells derived from the C57BL/6 mouse strain to generate $TNF^{-/-}$, $LT\alpha^{-/-}$, and $TNF/LT\alpha^{-/-}$ mice (Körner et al. 1997*a*; Riminton et al. 1998). This parental strain is susceptible to a form of EAE that is induced by injecting a fragment of the myelin oligodendrocyte glycoprotein (MOG), a minor component of CNS myelin.

TNF in Inflammatory Autoimmune Disease

In wild-type (WT) C57BL/6 mice, immunization with MOG leads to the development of clinical deficits, ranging from mild paralysis of the hind limbs to full quadriplegia. Clinical signs typically develop within 10–12 days after immunization, reaching a peak of severity by

day ~17. The disease remains at this high level for ~20 days and then resolves to a mild deficit by day ~40. $TNF^{-/-}$ mice develop clinical disease 5–7 days later than WT mice but then develop a severe clinical episode by day 20, with recovery at the same time as WT mice, despite the later onset (Körner et al. 1997*b*). Within the CNS of both WT and $TNF^{-/-}$ mice, a comparable level of demyelination occurs. Thus, even in the absence of TNF, clinical signs of EAE can develop, resolution of the disease appears normally, and demyelination proceeds. To understand the delayed onset of disease, we examined the CNS immunohistochemically and analyzed the population of inflammatory leukocytes in the CNS of both WT and $TNF^{-/-}$ mice at early stages after MOG inoculation. In WT animals at day 10–12, CNS perivascular inflammatory lesions are present, whereas the CNS of $TNF^{-/-}$ mice appeared uninflamed. However, leukocytes isolated from the whole CNS tissue are present in comparable numbers in both types of mice. Thus, substantial numbers of leukocytes accumulate in the $TNF^{-/-}$CNS but fail to form infiltrates within the CNS parenchyma. Precisely where these cells are distributed has not been defined, but localization to the perivascular space of capillaries and the meningeal vessels is likely. By day 20, when $TNF^{-/-}$ mice exhibit clinical signs of EAE, visible perivascular accumulations form. However, here also, leukocyte positioning is distinctive of WT mice, with cells forming tight aggregates around the microvasculature and with a remarkable lack of infiltration of the CNS parenchyma in the absence of TNF. Thus, throughout the immunologic process necessary for autoimmune inflammation in the CNS (see fig. 1), the only stage that is shown to be solely dependent on TNF is leukocyte movement within the tissue parenchyma. This is not to say that TNF cannot play a role in other steps of the inflammatory process; rather, TNF is not essential for those processes.

The conclusion from our EAE studies regarding a central role for TNF in leukocyte movement is strengthened by studies in $TNF^{-/-}$ mice, of pulmonary infection with *Mycobacterium tuberculosis* (Bean et al. 1999), NK cell localization within the peritoneum (Smyth et al. 1998), and lymphoid tissue integrity (Pasparakis et al. 1996; Körner et al. 1997*a*). In all cases, as in our work with the CNS, leukocytes fail to localize normally within inflamed tissues in the absence of TNF. This failure has significant consequences in each case. Although all leukocyte populations typically associated with the *M. tuberculosis*–infected lung are present in the $TNF^{-/-}$ lung, granulomas, which are necessary to remove bacilli, fail to form with their normal structure, and these mice show a greatly increased susceptibility to infection. NK cell development appears equivalent in WT and $TNF^{-/-}$ mice, and NK cells from the latter kill MHC class I negative tumors efficiently in vitro. Thus, TNF is not an essential cytotoxic effector molecule for NK cells, but, when the same tumors are instilled in the peritoneum of $TNF^{-/-}$ mice, the failure of NK cell localization permits tumor growth at that site. Finally, although all leukocyte populations in the spleen, lymph nodes, and Peyer's patches of $TNF^{-/-}$ mice are comparable in number and ratio to those of the WT, primary B cell follicles fail to form and follicular dendritic cells are not localized normally within the existing B cell area. The normal T cell–B cell regional demarcations are completely absent in Peyer's patches or lack precise definition in lymph node and spleen.

A prime candidate for the mediator of TNF-induced leukocyte trafficking is the chemokine family (also see Broide et al. 1999 [in this issue]). Chemokines control cellular interactions and movement in a precise and often cell-specific fashion and probably are important in all tissues and for the localization of many cell types, not only those of hematopoietic derivation. A simple explanation is that chemokine induction at a broad level requires TNF. Indeed, a role for TNF in induction or upregulation of leukocyte chemoattractants has been recognized for a decade, and the list of TNF-inducible chemokines is growing (Tessier et al. 1997). In the spleen, steady-state mRNA levels of a range of lymphocyte homing chemokines are reduced significantly in mice lacking TNF or TNF-R1 (Ngo et al. 1999). Furthermore, there is now abundant evidence that inflammation of the CNS is dependent on the induction of certain chemokines (Karpus and Ransohoff 1998), although it is not yet clear whether loss of TNF is sufficient to abolish the production of chemoattractants in CNS inflammation. Analyses similar to those conducted in the spleen (Ngo et al. 1999) must now be performed by means of inflamed CNS tissues from $TNF^{-/-}$ and WT mice. The evidence is far more direct in lung inflammation, in which production of the leukocyte chemoattractants MCP-1, MIP-2, and MIP-1α within the lung is compromised after treatment with anti-TNF antibody (Czermak et al. 1999).

TNF and LT Function in Autoimmune Inflammation: Inconsistencies and Controversies

The numerous studies, both clinical and experimental, showing inhibition of disease processes after TNF-blocking therapies argue for a significant role for TNF in promoting inflammation (reviewed in Feldmann et al. 1998). The mode of TNF action we have identified from studies in *TNF* gene-targeted mice is likely to be a preeminent one targeted by anti-TNF therapies. Conversely, there is evidence that TNF may contribute to resolution of inflammation (Cope 1998), but the mechanisms responsible for the effect and the range of circumstances in which it occurs remain undefined. However, it is disturbing to note the contrasting outcomes of EAE studies

in $TNF^{-/-}$ mice, including those that indicate a role for TNF in disease onset and acceleration (Körner et al. 1997*b*), or in disease inhibition (Liu et al. 1998), or that conclude, by inference from studies in LTα mice, that TNF has no role at all (Suen et al. 1997). These inconsistencies may be explained, at least in some cases, by inappropriate comparisons of mutated and WT mice, in which the differences between the two groups exceed the single mutation that is the object of the study. Thus, in one study, Kassiotis et al. (1999) introduced the $TNF^{-/-}$ mutation from 129 mice, which carry the H-2b haplotype, onto the EAE-susceptible SJL/J (H-2s) mouse strain, thereby effectively changing the MHC haplotype of the SJL/J mouse from s to b. To circumvent the difficulties caused by linkage between *TNF* and the other MHC genes, these authors used SJL/J-H-2b congenic mice as controls. After eliciting EAE by inoculating the animals with a CNS autoantigen to which SJL/J mice are susceptible, the authors found that $TNF^{-/-}$ mice show delayed disease onset and that inflammatory cuffing was more restricted to the immediate perivascular space (also see Körner et al. 1997*b*). Thus, when mutated and control mice are appropriately matched, TNF appears to be a consistent and significant factor in leukocyte motility during autoimmune inflammation.

Despite the difficulty of extracting clear answers about the role of TNF in these diseases, it may prove more challenging still to address the corresponding questions about the *LT*α and *LT*β genes. These genes are also tightly linked to other MHC loci, but, unlike *TNF,* they are also clearly implicated in lymphoid tissue neogenesis. Mice in which LTα1β2 -LTβR interactions are prevented by gene-targeting of ligand or receptor or by injecting specific inhibitors (Fu and Chaplin 1999) have neither lymph nodes nor Peyer's patches, and their splenic microarchitecture is substantially disturbed. In the spleen of these mice, as in $TNF^{-/-}$ mice, structural disorganization is associated with a reduction in expression of T and B cell homing chemokines (Ngo et al. 1999). Consistent with the extent of the phenotype, absence of LT signaling results in a more drastic loss of chemokine expression in the spleen than is seen in $TNF^{-/-}$ mice.

Data on the autoimmune responses of these mutant animals are inconsistent and difficult to interpret. In one study (Suen et al. 1997), $LT\alpha^{-/-}$ mice, which exhibit lymphoid tissue abnormalities, appeared poorly susceptible to EAE, relative to structurally normal, LTα-expressing WT mice. In another study of mice lacking both LTα and TNF, with one immunization regimen, the deficient mice were more severely affected than were the control, TNF/LTα–positive mice (Frei et al. 1997). Dissociating the consequences of functional LT absence (namely, the loss of lymphoid tissues) from a direct role of LT as an effector of the inflammatory process is difficult. Our own analyses indicate that, where structure is corrected but LT deficiency remains, mice become as susceptible to EAE as WT control mice (Riminton et al. 1998). Thus, controversy remains.

Therapeutic Opportunities and Experimental Challenges Ahead

The mechanism(s) by which in vivo TNF blockade induces a clear therapeutic benefit, particularly in RA and Crohn disease, remain(s) undefined (Feldmann et al. 1998; Hodgson 1999). Nevertheless, the success of this therapeutic modality indicates in an unequivocal way in humans, as opposed to animal model systems, the importance of TNF at key point(s) in inflammation and tissue damage. Whether diseases of this type reflect abnormalities/dysfunction specifically of the *TNF* gene or rather a necessary role for TNF in some other dysregulated pathway of inflammation requires further study. The outcome of animal model experiments discussed here indicate that the generation of chemokine gradients in tissues can be a TNF-dependent process and that subverting this probably contributes to the success of TNF-directed therapies. Hence, should future analysis of human inflammatory disease–susceptibility genes point to a genomic region that would include the TNF locus, associated evidence of dysregulated chemokine expression or disturbances in leukocyte movement may be a useful surrogate marker pinpointing specific effects to the *TNF* gene.

Separating the contributions of secreted LTα3 and TNF in inflammation remains problematic. Not only are the TNF and LT genes closely linked and not only may the loss of LT activities introduce confounding effects on lymphoid tissue integrity, but some effective therapeutics, such as TNFR-Ig fusion proteins, are capable of binding both proteins. Conversely, specific monoclonal antibodies neutralizing TNF but not LT are also highly effective in the treatment of RA and Crohn disease (Feldmann et al. 1998). The implication is that inhibiting TNF alone is sufficient. Whether there proves to be some advantage (or disadvantage) in additional neutralization of LT activity awaits further experience with TNF-specific vs TNF/LTα–blocking agents.

Finally, the relative importance of the membrane versus secreted forms of TNF in inflammation and tissue damage is an important issue, particularly given the view that drugs such as the TACE inhibitors, which prevent TNF cleavage from the cell surface, may be useful anti-inflammatories. That there could be no therapeutic effect or, worse, adverse consequences because of an increased concentration of biologically active TNF at the cell surface has not been fully appreciated or studied in any depth. At least two groups, our own included, have recently developed gene knock-in mice that express only a noncleavable form of TNF. The ability of membrane-

associated TNF alone to mediate the range of biological functions most commonly attributed to the secreted form of TNF should soon be documented in an in vivo system.

Acknowledgments

The authors thank Amy Beebe for critically reviewing the manuscript. The DNAX Research Institute is supported by Schering-Plough.

References

Bazzoni F, Beutler B (1996) The tumour necrosis factor ligand and receptor families. N Engl J Med 334:1717–1725

Bean AGD, Roach DR, Briscoe H, France MP, Körner H, Sedgwick JD, Britton WJ (1999) Structural deficiencies in granuloma formation in tumor necrosis factor gene-targeted mice underlie the heightened susceptibility to aerosol *Mycobacterium tuberculosis* infection which is not compensated for by lymphotoxin. J Immunol 162:3504–3511

Beutler B (1992) Tumor necrosis factors: the molecules and their emerging role in medicine. Raven Press, New York

Brinkman BMN, Huizinga TWJ, Kurban SS, Van der Velde EA, Schreuder GMT, Hazes JMW, Breedveld FC, et al. (1997) Tumour necrosis factor alpha gene polymorphisms in rheumatoid arthritis: association with susceptibility to, or severity of, disease? Br J Rheumatol 36:516–521

Brinkman BMN, Zuijdgeest D, Kaijzel EL, Breedveld FC, Verweij CL (1996) Relevance of the tumor necrosis factor alpha −308 promoter polymorphism in TNF alpha gene regulation. J Inflamm 46:32–41

Broide DH, Hoffman H, Sriramarao P (1999) Genes that regulate eosinophilic inflammation. Am J Hum Genet 65: 302–307 (in this issue)

Cope AP (1998) Regulation of autoimmunity by proinflammatory cytokines. Curr Opin Immunol 10:669–676

Czermak BJ, Sarma V, Bless NM, Schmal H, Friedl HP, Ward PA (1999) In vitro and in vivo dependency of chemokine generation on C5a and TNF-α. J Immunol 162:2321–2325

Feldmann M, Charles P, Taylor P, Maini RN (1998) Biological insights from clinical trials with anti-TNF therapy. Springer Semin Immunopathol 20:211–228

Frei K, Eugster H-P, Bopst M, Konstantinescu C, Lavi E, Fontana A (1997) Tumor necrosis factor α and lymphotoxin α are not required for induction of acute experimental autoimmune encephalomelitis. J Exp Med 185:2177–2182

Fu YX, Chaplin DD (1999) Development and maturation of secondary lymphoid tissue. Ann Rev Immunol 17:399–433

Galli-Taliadoros LA, Wood SA, Sedgwick JD, Körner H (1995) Gene knockout technology: a methodological overview for the interested novice. J Immunol Methods 181:1–15

Gerlai R (1996) Gene targeting studies of mammalian behaviour: is it the mutation or the background genotype? Trends Neurosci 19:177–181

Gruen JR, Weissman SM (1997) Evolving views of the major histocompatibility complex. Blood 90:4252–4265

Hodgson H (1999) Prospects of new therapeutic approaches for Crohn's disease. Lancet 353:425–426

Jacob CO, Fronek Z, Lewis GD, Koo M, Hansen JA, McDevitt HO (1990) Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor alpha: relevance to genetic predisposition to systemic lupus erythematosus. Proc Natl Acad Sci USA 87: 1233–1237

Karpus WJ, Ransohoff RM (1998) Chemokine regulation of experimental autoimmune encephalomyelitis: temporal and spatial expression patterns governing disease pathogenesis. J Immunol 161:2667–2671

Kassiotis G, Pasparakis M, Kollias G, Probert L (1999) TNF accelerates the onset but does not alter the incidence and severity of myelin basic protein-induced experimental autoimmune encephalomyelitis. Eur J Immunol 29:774–780

Kontoyiannis D, Pasparakis M, Pizarro TT, Cominelli F, Kollias G (1999) Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. Immunity 10:387–398

Körner H, Cook M, Riminton DS, Lemckert FA, Hoek RM, Ledermann B, Köntgen F, et al. (1997*a*) Distinct roles for lymphotoxin-α and tumor necrosis factor in organogenesis and spatial organization of lymphoid tissue. Eur J Immunol 27:2600–2609

Körner H, Riminton DS, Strickland D, Lemckert FA, Pollard J, Sedgwick JD (1997*b*) Critical points of tumor necrosis factor action in central nervous system autoimmune inflammation defined by gene targeting. J Exp Med 186: 1585–1590

Liu J, Marino MW, Wong G, Grail D, Dunn A, Bettadapura J, Slavin AJ, et al. (1998) TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination. Nat Med 4:78–83

Mackay F, Bourdon PR, Griffiths DA, Lawton P, Zafari M, Sizing ID, Miatkowski K, et al. (1997) Cytotoxic activities of recombinant soluble murine lymphotoxin-α and lymphotoxin-$\alpha\beta$ complexes. J Immunol 159:3299–3310

McDermott MF, Aksentijevich I, Galon J, McDermott EM, Ogunkolade BW, Centola M, Mansfield E, et al. (1999) Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. Cell 97:133–144

Ngo VN, Körner H, Gunn MD, Schmidt KN, Riminton DS, Cooper MD, Browning JL, et al. (1999) Lymphotoxin-$\alpha\beta$ and tumor necrosis factor are required for stromal cell expression of homing chemokines in B and T cell areas of the spleen. J Exp Med 189:403–412

Pasparakis M, Alexopoulou L, Episkopou V, Kollias G (1996) Immune and inflammatory responses in TNF-alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. J Exp Med 184:1397–1411

Peschon J, Torrance DS, Stocking KL, Glaccum MB, Otten C, Willis CR, Charrier K, et al. (1998) TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. J Immunol 160:943–952

Riminton DS, Körner H, Strickland DH, Lemckert FA, Pollard JD, Sedgwick JD (1998) Challenging cytokine redundancy: inflammatory cell movement and clinical course of experi-

mental autoimmune encephalomyelitis are normal in lymphotoxin-deficient but not TNF-deficient mice. J Exp Med 187:1517–1528

Sacca R, Cuff CA, Lesslauer W, Ruddle NH (1998) Differential activities of secreted lymphotoxin-alpha3 and membrane lymphotoxin-alpha1beta2 in lymphotoxin-induced inflammation: critical role of TNF receptor 1 signaling. J Immunol 160:485–491

Smyth MJ, Kelly JM, Baxter AG, Körner H, Sedgwick JD (1998) An essential role for tumor necrosis factor in natural killer cell-mediated tumor rejection in the peritoneum. J Exp Med 188:1611–1619

Stuber F, Udalova IA, Book M, Drutskaya LN, Kuprash DV, Turetskaya RL, Schade FU, et al. (1996) −308 tumor necrosis factor polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. J Inflamm 46:42–50

Suen WE, Bergman CM, Hjelmström P, Ruddle NH (1997) A critical role for lymphotoxin in experimental allergic encephalomyelitis. J Exp Med 186:1233–1240

Tessier PA, Naccache PH, Clark-Lewis I, Gladue RP, Neote KS, McColl SR (1997) Chemokine networks in vivo: involvement of C-X-C and C-C chemokines in neutrophil extravasation in vivo in response to TNF-alpha. J Immunol 159:3595–3602

Theofilopoulos AN (1995) The basis of autoimmunity: part II genetic predisposition. Immunol Today 16:150–159

Vassalli P (1992) The pathophysiology of tumor necrosis factors. Ann Rev Immunol 10:411–452

Wilson AG, Di Giovine FS, Duff GW (1995) Genetics of tumour necrosis factor-alpha in autoimmune, infectious, and neoplastic diseases. J Inflamm 45:1–12

Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW (1997) Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 94:3195–3199

Zhang M, Tracey KJ (1998) Tumor necrosis factor. In: Thomson AW (ed) The cytokine handbook. Academic Press, London, pp 517–548