level production of TNF may contribute to the inflammatory cardiovascular collapse. By contrast, chronic, low-grade inflammatory cytokines. These effects culminate in apoptosis of lymphocytes, derangement of the immune response, or autoimmune disease.

All members of the TNF-ligand family are believed to consist of three polypeptide chains. All but lymphotoxin-β (which consists of a single lymphotoxin-α subunit and two lymphotoxin-β subunits) are made up of three identical subunits. All except lymphotoxin-α (which is entirely secreted) and TNF (which is predominantly secreted) are transmembrane proteins that act chiefly through cell-to-cell contact. Nerve growth factor, a dimeric protein, is not actually a member of the TNF-ligand family. Rather, it was apparently adapted in the course of evolution to serve its receptor, a true member of the TNF-receptor family.

All members of the TNF-receptor family are believed to be transmembrane proteins that consist of two identical subunits. The family is defined by a cysteine-rich domain that activates a corresponding family of structural- and signal-transducing subunits. All but lymphotoxin-α and the receptors can cause characteristic disturbances of lymphocytes, derangement of the immune response, or autoimmune disease.

Because it proved to be highly toxic in animals and humans, TNF did not fulfill initial expectations that it would be useful in the treatment of cancer. However, considerable evidence suggests that overproduction or inappropriate production of TNF may play a part in various chronic inflammatory diseases. Produced largely by macrophages in response to inflammatory stimuli such as lipopolysaccharide, TNF binds to receptors present on virtually all cells throughout the body. TNF, if released systemically in large amounts all at once, modifies the anticoagulant properties of endothelial cells, activates neutrophils, and induces the release of other inflammatory cytokines. These effects culminate in cardiovascular collapse. By contrast, chronic, low-level production of TNF may contribute to the inflammatory response. Bone resorption, fever, anemia, and wasting may all, in some measure, be attributable to TNF (Fig. 1).

What beneficial functions does TNF have? Can its activities be blocked, and if so, at what risk? Above all, how does TNF work? Answers to each of these questions have begun to emerge. They point to regulatory mechanisms that control the biosynthesis of TNF, address the molecular reactions that permit TNF to mediate cell signaling, and suggest a practical means of blocking the activity of TNF for therapeutic effect.

**THE TNF-LIGAND AND TNF-RECEPTOR FAMILIES**

TNF is one of 10 known members of a family of ligands that activate a corresponding family of structurally related receptors (Table 1). The receptors initiate signals for cell proliferation and apoptosis (programmed cell death). These signals are required for the normal development and function of the immune system. Excessive signaling through some of the receptors can cause severe inflammatory reactions, tissue injury, and shock. Mutations of the genes corresponding to the ligands or the receptors can cause characteristic disturbances of lymphocytes, derangement of the immune response, or autoimmune disease.

All members of the TNF-ligand family are believed to consist of three polypeptide chains. All but lymphotoxin-β (which consists of a single lymphotoxin-α subunit and two lymphotoxin-β subunits) are made up of three identical subunits. All except lymphotoxin-α (which is entirely secreted) and TNF (which is predominantly secreted) are transmembrane proteins that act chiefly through cell-to-cell contact. Nerve growth factor, a dimeric protein, is not actually a member of the TNF-ligand family. Rather, it was essentially adapted in the course of evolution to serve its receptor, a true member of the TNF-receptor family.

All members of the TNF-receptor family are believed to be transmembrane proteins that consist of two identical subunits. The family is defined by a cysteine-rich amino-acid motif that recurs three to six times in the extracellular domain. The cytoplasmic domains vary more than the extracellular domains. Notably, certain receptors contain a 60-residue cytoplasmic sequence known as the “death domain.” In the 55-kd TNF receptor and the Fas receptor, this domain is required for the transduction of an apoptotic signal.

With the exceptions of TNF and lymphotoxin-α, each member of the ligand family binds to a specific receptor. TNF and lymphotoxin-α engage two receptors (the 55-kd and 75-kd TNF receptors) with similar affinity. These two cytokines initiate similar (if not identical) biologic responses, although they are produced by different types of cells (lymphotoxin-α is produced exclusively by lymphocytes and natural killer cells, and TNF predominantly by macrophages) in response to different stimuli (antigenic or mitogenic stimuli for lympho-
toxin-α and lipopolysaccharide or other macrophage-activating agents for TNF).

**FUNCTIONS OF TNF LIGANDS AND RECEPTORS**

For many years, the role of members of the TNF-ligand and TNF-receptor families in immunity and in the development of the immune system remained speculative. The first indication of their function came from the finding that mutations of the ligands or their receptors can cause disease. Striking examples are the 55-kd and the 75-kd TNF receptors. Both the 55-kd and the 75-kd TNF receptors have been identified as models of systemic lupus erythematosus because they have lymphadenopathy and splenomegaly and form autoantibodies. In the homozygous state, each mutation causes the accumulation of large numbers of T cells lacking the CD4 and CD8 surface proteins. Heterozygous pairing of the 55-kd and the 75-kd TNF receptors, suggesting that the products of the two loci might interact with one another.

Further study demonstrated that the IgM antibodies to the production of IgG antibodies and clonal expansion of antigen-reactive B cells. In mice, deletion of the CD40-ligand or CD40-receptor genes results in a phenotype that resembles the disease that occurs in humans.

Since naturally occurring mutations that interfere with the function of TNF, lymphotxin-α, or their two receptors have not been identified, it has been necessary to ablate the genes in mice through a “gene knockout.” Both the 55-kd and the 75-kd TNF receptors have been deleted in this manner. Genetically engineered mice lacking the 55-kd TNF receptor are moderately resistant to the lethal effect of lipopolysaccharide but highly susceptible to infection by *Listeria monocytogenes*. Mice lacking the 75-kd TNF receptor are moderately resistant to the lethal effect of TNF itself and to dermal necrosis elicited by repeated intradermal injections of TNF.

**Figure 1. Range of Actions of TNF.**

In response to inflammatory stimuli such as lipopolysaccharide, macrophages produce TNF. TNF binds to receptors present on virtually all cells throughout the body, causing a variety of reactions.
These results may reflect the fact that the lympho-
 toxin-α gene is required for the formation of not only the lympho-
toxin-α homotrimer but also the lympho-
toxin-β heteromer. 
13 Interaction between the lympho-
toxin-β heteromer and the lympho-
toxin-β receptor is proba-
bly required for lymph-node development. 
17,44,45 Lymph 
nodes fail to develop in lympho-
toxin-α−deficient mice despite the fact that the lymphocyte subtypes are di-
istributed normally in peripheral blood. Lympho-
toxin-α−deficient lymphocytes are readily incorporated into the 
lymph nodes of normal mice, and lympho-
toxin-α−positive lymphocytes fail to induce the development of 
lymph nodes in lympho-
toxin-α−deficient mice. There-
fore, the failure of lympho-
toxin-β heteromer to be ex-
pressed on the surface of a nonlymphoid cell, such as a 
lymph-node stromal element, would seem to preclude 
normal development of peripheral lymphoid tissues. 
46

**HOW THE RECEPTORS WORK**

Interactions between TNF and its receptors are 
presumably typical of interactions between the other lig-
and–receptor pairs in these two molecular families. 
Trimeric ligands of the TNF family may cause aggre-
gation or clustering of receptor subunits, thereby trig-
gering a cellular response, since antibodies against ei-
ther of the two TNF receptors mimic the actions of 
TNF. 
14 Support for the aggregation model (Fig. 2A) 
is bolstered by the crystal structure of lympho-
toxin-α, which forms a complex with extracellular-domain 
fragments of the 55-kd TNF receptor, 
19 in which three re-
ceptor fragments crystallize with each lympho-
toxin-α trimer.

Crystallization of the extracellular domain of the 55-
kid receptor in the absence of ligand yields a dimeric 
protein, in which each subunit is arranged head to head with the 
other. The 55-kd receptor on the cell surface 
might thus be dimeric in the absence of TNF. A dimeric 
conformation would allow for a hexagonal array of dimeric receptors and trimeric ligand molecules, which could generate signals by making contacts between their cytoplasmic domains (Fig. 2B). Alternatively, each dimeric receptor might act as an independent “molecular switch,” 
undergoing rearrangement after engaging the 
trimeric ligand 
50 (Fig. 2C).

The molecular-switch model is strongly supported by the 
effect produced by substituting an erythropoietin-
receptor extracellular domain for the extracellular 
domain of either TNF receptor. This leads to constitutive 
signaling activity in the cell (i.e., a perpetual “on” 
state).

20 The conformation of the receptor dimer, rather than dimerization itself, is thus the critical issue in signal 
transduction. Moreover, an essential attribute of the 
extracellular domain of the TNF receptor is its ability to 
prevent signal transduction in the absence of a lig-
and. If the extracellular domains are removed, 
31 grossly modified, 
30 or displaced by an antibody, 
47,48 signaling is initia-
ted.

One of the most intriguing actions of TNF is the in-
duction of apoptosis. Apoptosis is almost certainly re-
vant to some of the toxic effects of TNF, such as shock

---

### Table 1. Recently Characterized Members of the TNF-Ligand and TNF-Receptor Families.*

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Source of Ligand</th>
<th>Receptor</th>
<th>Distribution of Receptors</th>
<th>Ability to Initiate Apoptosis</th>
<th>Cytoplasmic Mediators</th>
<th>Mutation or Knockout Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF and lymphotixin-α</td>
<td>TNF: macrophages, lymphocytes, keratinocytes, others</td>
<td>55-kd TNF receptor</td>
<td>Many cells</td>
<td>Yes (strong)</td>
<td>TRADD, TRAP-1</td>
<td>Both TNF and lymphotixin-α: absent lymph nodes, decreased lipopolysaccharide responses; failure to contain listeria or mycobacteria infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T cells</td>
<td>75-kd TNF receptor</td>
<td>Many cells</td>
<td>Yes</td>
<td>TRAF-1, TRAF-2</td>
</tr>
<tr>
<td>Lymphotixin-β heteromer</td>
<td>T cells, others</td>
<td>Lymphotixin-β receptor (TNF-receptor–related protein)</td>
<td>T cells, B cells, others</td>
<td>Yes</td>
<td>LAP-1 (CRAF-1)</td>
<td>ND</td>
</tr>
<tr>
<td>Fas ligand</td>
<td>T cells</td>
<td>Fas receptor</td>
<td>Many cells</td>
<td>Yes (strong)</td>
<td>Tyrosine phosphatase (FAP-1), TRADD, FADD (MORT-1)</td>
<td>ND</td>
</tr>
<tr>
<td>Nerve growth factor CD40 ligand</td>
<td>NA</td>
<td>Nerve growth factor receptor</td>
<td>Neurons, B cells, T cells</td>
<td>No</td>
<td>CRAF-1, CAP-1</td>
<td>X-linked immunodeficiency with increased IgM and decreased or absent IgG, IgA, IgD</td>
</tr>
<tr>
<td>CD27 ligand</td>
<td>T cells</td>
<td>CD27</td>
<td>T cells</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD30 ligand</td>
<td>T cells</td>
<td>CD30</td>
<td>T cells</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>OX-40 ligand</td>
<td>T cells</td>
<td>OX40</td>
<td>T cells</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4-1BB ligand</td>
<td>T cells</td>
<td>4-1BB</td>
<td>T cells</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Other members of these two families include the CD27, CD30, OX-40, and 4-1BB ligands and receptors. TRADD denotes TNF-receptor–associated death domain, TRAP-1 TNF-receptor–associated factor 1, LAP-1 latent membrane protein type 1–associated protein, CRAF-1 CD40-receptor–associated factor 1, ND not determined, FAP-1 Fas-associated protein 1, FADD (or MORT-1) Fas-associated death domain, NA not applicable, and CAP-1 CD40-associated protein 1.
and inflammation. TNF-induced apoptosis may also have physiologic relevance, as does the apoptosis induced by the Fas receptor. It is possible, for example, that TNF-mediated apoptosis of infected cells helps protect the host. It is likely that both TNF receptors participate in cell death, although the 55-kd receptor is more potent than the 75-kd receptor. Since the cytoplasmic domains of the two receptors are structurally different, each must initiate apoptosis through distinct mechanisms.

Proteins Used by the TNF-Receptor Family for Signal Transduction

Several proteins that bind intracellularly to receptors of the TNF family have been identified (Fig. 3). The first to be identified were the TNF-receptor–associated factors (TRAFs), which have high affinity for the 75-kd TNF receptor. Their biologic function is unknown. Most contain two protein motifs termed the “zinc finger” and “ring finger.” TRAF-2 may form a homodimer with itself or a heterodimer with TRAF-1. TRAF-2, TRAF-3 (also known as CD40-receptor–associated factor 1 [CRAF-1] or latent membrane protein type 1–associated protein [LAP-1]), and the closely similar CD40-associated protein 1 (CAP-1) bind not only to the 75-kd TNF receptor, but also to the lymphotoxin-receptor and the CD40 receptor. Moreover, TRAF-3 also binds to latent membrane protein type 1, a protein of the Epstein–Barr virus that is essential to cell transformation.

An entirely different class of cytoplasmic proteins bind to the 55-kd TNF receptor and the Fas receptor. These proteins are important in transducing signals for programmed cell death. The Fas–associated death domain (FADD), also called MORT-1, the TNF-receptor–associated death domain (TRADD), and the receptor interacting protein (RIP) bind to the Fas receptor, the 55-kd TNF receptor, and both receptors, respectively. Each of these proteins contains a version of the death domain found within the receptors them-
selves. This motif permits interaction between receptor and transducer molecules.

FADD\textsuperscript{24,52} is a proximal transducer of the apoptotic activity of the Fas receptor, with which it forms a heterodimer. Engagement of the ligand causes the release of homodimeric FADD, which relays the death signal to the cytoplasm. FADD is incapable of forming heterodimers with receptors encoded by the mutant \textit{lpr} \textit{CG} gene. Hence, it is at precisely this level that the \textit{lpr} \textit{CG} mutation interrupts signaling.

Acting in an analogous fashion, TRADD is a proximal transducer of apoptosis mediated through the 55-kd TNF receptor.\textsuperscript{14} RIP appears to serve both the 55-kd TNF receptor and the Fas receptor. The transducers may interact with distinct targets downstream from the receptor. Although overexpression of any of the transducers can initiate cell death, different portions of each protein act to carry the apoptotic signal forward within the cytoplasm.\textsuperscript{23} Moreover, RIP is far larger than either TRADD or FADD and contains a kinase domain.\textsuperscript{54}

In accordance with the molecular-switch hypothesis, a receptor modified by the binding of ligand might effectively catalyze the formation of homodimeric FADD, TRADD, or RIP (Fig. 4), causing conformational changes in these molecules that lead to further reactions in the signaling cascade. This scenario is consistent with the observation that modified receptors are constitutively activated in the absence of ligand.\textsuperscript{50} A detailed discussion of the distal components of the cascade by which the death signal is conveyed is beyond the scope of this review.\textsuperscript{55-58}

**Clinical Effects of TNF and Lymphotoxin-α Blockade**

Extensive clinical trials have been performed to test monoclonal antibodies that selectively neutralize TNF (but not lymphotoxin-α) in the treatment of septic shock. To date, double-blind, controlled studies have not documented a substantial benefit.\textsuperscript{59,60} However, septic shock is a fulminant disease in which considerable
pressed at very high levels in the cell. A and B refer to the sub-
pathway that results in cell death. Homodimers do not form
nal transduction to be carried downstream to components of the
plasmic domain of a dimeric receptor. The relation between the
2C, ligand binding causes a conformational change in the cyto-
triple switch model presented in Figure 2C, ligand binding causes a conformational change in the cyto-
plasmic domain of a dimeric receptor. The relation between the
domains (red rectangles) then favors the formation of
TRADD, RIP, or FADD (or MORT-1) homodimers, permitting sig-
formation of TNF protein increases by a factor of
molecular-switch model presented in Figure 2C, ligand binding causes a conformational change in the cyto-
TNF, it is fortunate that the groundwork for a compre-
hensive understanding of this cytokine has been laid
The breadth of actions ascribed to TNF is remark-
able (Fig. 1). The molecule is one of the best-character-
zation and translation in vivo in activating TNF?
The lupus-like state that follows abrogation of the
complications, however. First, anti-TNF antibodies do not
neutralize both TNF and lymphotoxin-α. Second, the antigenicity of murine monoclonal antibod-
ies, and even humanized monoclonal antibodies, may
preclude long-term therapy. Third, complement fixation
other reactions related to the formation of immune complexes might be harmful in patients receiving
anti-TNF antibodies. Finally, the concentration of mon-
oclonal anti-TNF antibody required to achieve neutral-
ization is very high; consequently, blockade might be
The use of chimeric inhibitor molecules (Fig. 5), in
which the extracellular domain of the TNF receptor is
spliced to an immunoglobulin heavy-chain fragment, might circumvent all these problems. Such molecules
are as stable in vivo as immunoglobulins. Because they
are composed of two nonantigenic elements, they are
minimally antigenic. Their mode of action is highly specific, since their binding domain is a receptor. More-
over, they are broad-spectrum agents, since they neu-
tralize both TNF and lymphotoxin-α, preventing the activation of both TNF receptors. On a weight basis,
chimeric inhibitors are far more potent than monoclon-
al anti-TNF antibodies, because the receptor has a far
higher affinity for the ligand than does the antibody.

Chimeric TNF inhibitors have been produced in mice
with adenoviral vectors. Milligram quantities of the protein were present per milliliter of plasma, causing
complete neutralization of TNF and lymphotoxin-α in vivo. One day, gene transfer might be used to produce
the inhibitor protein in patients with inflammatory dis-
and tissue injury. Low levels of TNF may account for
against intracellular pathogens. It is a pro-inflammatory
mediator that can, when overproduced, cause shock and tissue injury. Low levels of TNF may account for
the state of insulin resistance that contributes to the de-
velopment of type II diabetes mellitus. Given the
complexity of the biomedical problems that involve
TNF, it is fortunate that the groundwork for a com-
prehensive understanding of this cytokine has been laid
with the identification of its receptors and many of their
signaling intermediates.

The lupus-like state that follows abrogation of the
function of the Fas ligand or receptor suggests that
some autoimmune disorders could involve defects in the Fas- or TNF-signaling axes. The observation that the
administration of TNF attenuates or prevents some au-
toimmune diseases in animals supports this view. TNF may never prove useful in the treatment of widely
 disseminated cancer, but the insight into tumor-cell vul-
nerness gained through studies of TNF signal trans-
duction may ultimately yield novel chemotherapeutic
approaches.

Ways to block the biosynthesis or action of TNF
could have important clinical applications. TNF has
served as the principal end point in most studies of en-
dotoxin signal transduction. It is likely that drugs im-
pairing each step of that process will soon be tested for
antiinflammatory efficacy. TNF and lymphotoxin-α can
already be neutralized, and neutralization of other mem-
ers of the ligand family is being explored. Thus, new
and highly specific approaches to the treatment of in-
flammatory disease may soon be at hand.

**DISCUSSION**

**DR. JEFFREY FLIER:** What is the relative role of tran-
scription and translation in vivo in activating TNF? Can you discuss your work on transgenic mice that re-
lates to this issue?

**DR. BEUTLER:** The biosynthesis of TNF is controlled
by two switches — one transcriptional and one trans-
lational — that work in concert with each other. The
activation of macrophages by lipopolysaccharide caus-
es a 30-fold increase in TNF messenger RNA and a
100-fold increase in translational efficiency. The rate
of production of TNF protein increases by a factor of
several thousand. My colleagues and I created trans-
genic mice bearing a reporter construct in which the
TNF coding sequence was replaced by DNA coding...
for the marker enzyme chloramphenicol acetyltransferase, or CAT. The CAT-reporter transgene behaved rather like the TNF gene itself and revealed to us that TNF is produced constitutively in the thymus and trophoblast. However, its function in these tissues remains unknown.

A PHYSICIAN: What signals do the ring- and zinc-finger proteins convey?

DR. BEUTLER: There is a tendency to think that they may transduce mainly proliferative signals. The receptors to which they bind have largely proliferative functions. This is true of the 75-kd TNF receptor and the CD40-ligand receptor. It may also be true of the lymphotoxin-α receptor. Common transducers may therefore produce a common effect, in what amounts to cross-communication among the three. On the other hand, each receptor does have unique properties, and it is unlikely that any one of them can truly substitute for another.

A PHYSICIAN: What do we know about how TNF actually causes apoptosis? Specifically, what makes tumor cells so sensitive to it?

DR. BEUTLER: It is still not clear why the cells are so sensitive. Almost any kind of cell can be lysed by TNF in the presence of protein-synthesis inhibitors. The general thinking has been that tumor cells lack a short-lived protective factor that enables normal cells to escape the cytolytic effect of TNF. Members of the family of proteases containing interleukin-1β-converting enzyme have been implicated as downstream transducers in the TNF and Fas signaling pathways. Inhibitory members of the Bcl-2 family of proteins, such as Bak, may also be involved. However, a clear and complete biochemical pathway has yet to be established. Therefore, we cannot say precisely why certain tumor cells are sensitive to TNF, nor do we know what advantage this might confer.

DR. VIKAS SUKHATME: Is it possible to make TNF antagonists?

DR. BEUTLER: True antagonists have not yet been designed, although theoretically it should be possible to achieve this. A heteromeric form of TNF, with only one site capable of binding to the receptor, might fulfill such a function.

DR. FLIER: Could you tell us whether the pharmaceutical industry is using the TNF inhibitors that you developed?

DR. BEUTLER: The chimeric inhibitors have now been produced for clinical use by a number of companies. They are being tested for efficacy in the treatment of various diseases.
of rheumatoid arthritis. Already, proof of principle has been supplied by the striking remissions induced by anti-TNF antibodies. There is reason to believe that chimeric inhibitors will perform even better, given their activity against lymphotixin-α as well as TNF, their extraordinary affinity for these ligands, and their relative lack of antigenicity.

**References**


