Immunoregulation

Introductory article

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Article Contents

The immune system serves essential functions in protection from numerous pathogenic organisms and, in general, is not harmful to the host. The process by which the immune response is restrained or controlled is termed immunoregulation. A number of different aspects of the immune system contribute to this process of immunoregulation, some of the most important being signals from antigen-presenting cells by costimulatory molecules, the effects of cytokines and apoptotic cell death.

Introduction

Immune responses are essential in providing protection from infectious organisms such as bacteria, viruses and parasites. Not surprisingly, the immune response is finely tuned to respond rapidly and appropriately to these agents. However, not only must the response be turned on quickly, but, equally important, it must be turned off effectively to prevent the harmful effects of an unchecked immune response. Thus, there are many steps at which the immune response is regulated. From the initial phases in which lymphocytes encounter antigen to the waning of the immune response after an infection, there is a variety of steps during the immune response that are targets for control; like many other systems in the body, homeostasis of the immune response is of great importance.

Not only must the immune response to exogenously delivered antigens be controlled, but the immune response to self antigens also must be constrained or 'turned off'. Therefore, a fundamental feature of the immune system is the lack of responsiveness to self antigens. This unresponsive state is termed tolerance. Although we do not have a full understanding of this process, recent advances in elucidating the molecular basis of immunoregulation have provided additional insights into how tolerance may be achieved.

The fundamental step in the specific immune response, namely how lymphocytes develop and what they 'see' as foreign (compared to self), is governed by processes known as positive and negative selection. For T cells this occurs primarily in the thymus. Self-reactive lymphocytes are eliminated by negative selection, also termed clonal deletion, and genetic determinants such as major histocompatability complex (MHC) molecules and T-cell receptor (TCR) molecules, are key elements. The elimination of self-reactive lymphocytes in the thymus is denoted as central tolerance. Lymphocyte selection, although exceedingly important, will not be discussed further in this article.

Other critical means by which the immune response is controlled include: signalling by antigen, inhibitory and costimulatory receptors, cytokines and cell death (**Figure 1** and **Table 1**). These mechanisms contribute to what has been termed peripheral tolerance and will be the major focus of this article. Many of these processes apply to both T and B cells, but in general the events in T cells are better characterized and will be emphasized. How these regulatory mechanisms contribute to protection from infectious organisms and what occurs when there is a failure of immunological regulation will be considered, as will new therapeutic approaches based on immunoregulatory principles.

Physiological Immunoregulation

Lymphocyte activation: antigen receptors, inhibitory receptors and costimulatory molecules

Recognition of foreign antigen by lymphocytes initiates a cascade of biochemical steps that lead to cellular activation. In contrast, variant peptide antigens that bind T-cell antigen receptors less well do not fully activate lymphocytes, leading to partial activation and altered substrate phosphorylation. These peptides are termed altered peptide ligands and can cause anergy or nonresponsiveness of T cells.

A specialized motif found in several immune receptors and in antigen receptors is the immunoreceptor tyrosine-based activation motif (ITAM), which serves to recruit other tyrosine kinases. Importantly, in addition to activating receptors, a number of immune inhibitory receptors have been identified. Instead of ITAMs, these receptors have immunoreceptor tyrosine-based inhibitory motifs (ITIMs) which recruit tyrosine phosphatases.

Such receptors were first recognized in natural killer (NK) and B cells, but are also expressed in T cells and macrophages. This is one way in which activation through antigen receptors on lymphocytes is carefully regulated.

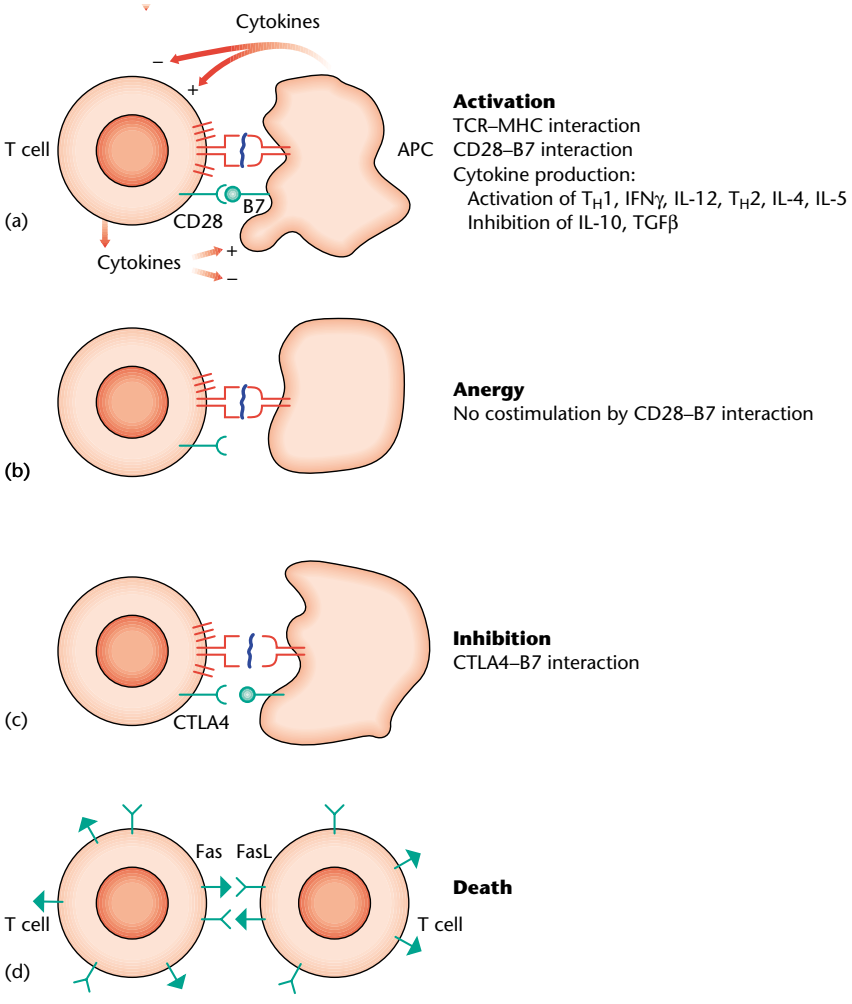

Figure 1 Mechanisms that regulate immune responses. (a) Foreign antigens are presented to T cells by antigen-presenting cells (APCs) such as macrophages, B cells and dendritic cells. T cells recognize foreign antigen by virtue of their clonotypic T-cell antigen receptors (TCRs). Occupancy of the TCR initiates a series of biochemical events that lead to activation of T cells; however, the T cells are not fully activated unless they also receive signals from costimulatory molecules like CD28, which binds to B7 molecules on APCs. When fully activated, T cells produce cytokines, some of which enhance inflammatory and immune responses whereas others inhibit. Still others regulate the type of immune response, promoting allergic or cell-mediated responses. (b) If T cells do not receive signals from CD28, and if they are unable to produce and respond to cytokines, they may become anergic or nonresponsive. (c) In addition, after activation T cells upregulate another molecule, cytotoxic T lymphocyte-associated antigen 4 (CTLA4) which also binds B7 molecules, in contrast to CD28, CTLA4 transmits signals that inhibit lymphocyte activation. (d) When activated, T cells also express the molecules Fas and Fas ligand (FasL). Interaction of these molecules causes lymphocytes to undergo apoptotic cell death, thus constraining immune responses. Lymphocytes can also die because of the lack of cytokines; this is termed cytokine withdrawal apoptosis. IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; TGF, transforming growth factor; T_H, T helper cell.

Importantly, though, signals from antigen receptors are not sufficient to activate lymphocytes. Recall that T cells only bind antigens that are 'presented' by other cells. Thus, another key aspect of T-cell activation is engagement of molecules on the surface of the antigen-presenting cell (APC), called costimulatory molecules. Increasingly it is being recognized that APCs, such as dendritic cells, have pivotal roles in activating or tolerizing lymphocytes. Among the best characterized molecules in this category are members of the B7 family (B7-1 (CD80), and B7-2 (CD86)). These molecules can be upregulated by infectious organisms and by cytokines; engagement of B7-1 and B7-2 by a counterreceptor on T cells, CD28, greatly enhances activation signals. Significantly, activation of T cells without a costimulatory signal can lead to anergy. Clonal anergy, then, in addition to clonal deletion is another means to achieve nonresponsiveness or tolerance to a given antigen.

It has long been recognized that if adjuvants – substances that elicit inflammation (such as mycobacterial

Table 1 Major mechanisms involved in immunoregulation

Mechanism	Effect	Comment
Antigen receptor signalling	Positive	Activates kinases Altered peptides can inhibit signalling
Inhibitory receptors	Negative	Bind phosphatases
Costimulatory molecules on antigen-presenting cell		Upregulated by inflammation
B7/CD28	Positive	T-cell counterreceptor CD28
B7/CTLA4	Inhibitory	CTLA-4 T-cell counterreceptor upregulated by activation; also binds B7
Cytokines	Positive or negative	Numerous
Apoptosis	Inhibitory	Two forms: cytokine withdrawal and activation-induced cell death

CTLA4, cytotoxic lymphocyte-associated antigen 4.

cell wall constituents) – are coadministered with antigen, a vigorous immune response is elicited. It is now clear that adjuvants increase the expression of costimulatory molecules on APCs, permitting full lymphocyte activation. Inflammation can thus provide a 'danger' signal to the immune system, communicating the threat of pathogenic challenge to lymphocytes. Conversely, in the absence of inflammation, antigen may be presented to T cells by cells with poor expression of costimulatory molecules; this may lead to anergy of the T cells.

It should be noted that there is also a second T-cell counterreceptor for B7-1 and B7-2 called cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). In contrast to CD28, CTLA-4 transmits signals that inhibit lymphocyte activation, providing a pathway of classical feedback inhibition.

Costimulation can work by activating both APCs and lymphocytes. An example of this is CD40 (on APCs) and CD40 ligand (on activated T cells).

In summary, lymphocytes have antigen receptors that transmit activation signals, but full activation requires the engagement of costimulatory molecules; in their absence lymphocytes may be anergized. This may be one mechanism for extrathymic or peripheral tolerance. In addition, antagonizing activation signals are the signals provided by inhibitory receptors. Thus, even at the earliest steps in lymphocyte activation, there are opposing signals that constrain activation.

Cytokines

After foreign antigens presented by activated APCs have initiated lymphocyte activation, the lymphocytes in turn begin to produce soluble factors termed cytokines or interleukins. These are factors that act on lymphocytes and other cells and thus are major regulators of the immune response.

Cytokines involved in innate immunity and inflammation

The major proinflammatory cytokines include: interferon (IFN) α/β, interleukin (IL)1, tumour necrosis factor (TNF) α, TNFβ or lymphotoxin (LT), IL-6 and the chemokines. Interferons inhibit viral replication and cellular proliferation, upregulate major histocompatibility complex (MHC) class I and downregulate of class II expression.

IL-1 is a major inflammatory cytokine whose principal effects are: the induction of fever, acute-phase protein synthesis and cachexia (wasting). It also induces production of other cytokines including: TNFα, IL-6 and chemokines. It is induced by lipopolysaccharide, TNFα and IL-1 itself.

The actions of TNFα are similar, in some respects, to those of IL-1 and include: induction of fever, hypoglycaemia, cachexia and the acute-phase response in the liver; activation of the coagulation system; and increased adhesion of cells to endothelium. TNFα also causes depression of cardiac contractility and reduction of vascular resistance and, as such, is the major mediator of septic shock. Furthermore, it upregulates MHC class I expression, activates phagocytes and induces mononuclear phagocytes to produce cytokines such as IL-1, IL-6 and chemokines.

A factor closely related to TNFα is lymphotoxin (LT; also called TNFβ), which competes with TNFα for binding to the same receptors. As expected, LT has many of the same effects as TNFα but, in addition, it can bind to another protein, LTβ, to form a cell surface complex. LTβ knockout mice have severe disruption of lymph node architecture, indicating that LT and LTβ have important functions in regulating lymph node structure. LT, like TNFα, is encoded within the MHC and is produced by activated T cells (see below).

Another major inflammatory cytokine is IL-6, which acts on the liver to induce production of the acute-phase proteins (C-reactive protein, serum amyloid A,

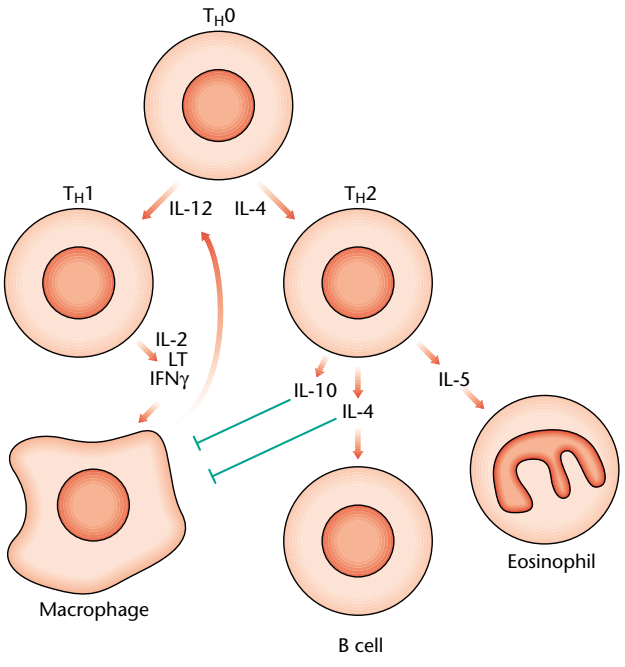

Figure 2 Immunoregulation and the T_H1–T_H2 paradigm. Undifferentiated helper T cells (T_H0) only produce interleukin (IL)-2. IL-12, produced by macrophages in response to pathogens, drives T_H1 differentiation. In conjunction with IL-18, IL-12 causes interferon (IFN) γ production, which further activates monocytes. When differentiated, T_H1 cells produce IL-2, IFNγ and lymphotoxin (LT). Among their actions, these cytokines serve to activate macrophages further. In contrast, T_H2 cells produce IL-4, IL-5 and IL-10. IL-4 inhibits macrophage activation, causes class switching so that B cells produce immunoglobulin E, and is a growth factor for mast cells. IL-5 activates eosinophils and IL-10 inhibits immune responses.

α_2-macroglobulin and fibrinogen) and to decrease synthesis of albumin and transferrin. In addition, IL-6 is a growth and differentiation factor for B cells. IL-6 is produced by a variety of cells including mononuclear phagocytes in response to IL-1 and TNFα.

Chemokines are a large family of *chemo*tactic cyto*kines*, which attract various types of leucocytes including T cells, neutrophils and mononuclear cells.

Immunoregulatory cytokines

The major immunoregulatory cytokines include: IFNγ, IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-15 and IL-18. These cytokines can dramatically affect not only the strength of the immune response but also its character.

Differentiation of T helper cells types 1 and 2

A critical concept that has emerged is that precursor CD4 + or helper T cells differentiate to one of two effector phenotypes (**Figure 2**). In mice, T helper type 1 (T_H1) cells drive the immune response towards a cell-mediated immune response whereas T helper type 2 (T_H2) cells promote a humoral or allergic response. T_H1 cells produce IL-2, LT (TNFβ) and IFNγ, whereas T_H2 cells produce IL-4, IL-5 and IL-13. As discussed below, T_H1 responses are important for clearing the majority of different types of infectious organisms (viruses, bacteria, intracellular parasites, fungi), whereas T_H2 responses play a significant role in mediating the response to multicellular and extracellular parasites.

Precisely how T_H1 versus T_H2 differentiation occurs is incompletely understood. A number of transcription factors such as GATA-3, nuclear factor of activated T cells and cMaf are evidently involved. It is also clear that cytokines themselves are important in this process, the most important being IL-12 and IL-4; IL-12 is a major factor that stimulates the differentiation of precursor CD4 + cells toward a T_H1 phenotype, whereas IL-4 appears to play the key role in T_H2 subset differentiation.

IL-2 is an autocrine T-cell growth factor required for progression of T cells through the cell cycle. It also augments the cytolytic activity of T and NK cells and induces IFNγ secretion in NK cells. It is a growth factor for B cells, induces immunoglobulin class switching, and also activates macrophages. Thus IL-2 is an important factor which enhances the magnitude of the immune response. Interestingly, though, IL-2 and IL-2 receptor knockout mice manifest unrestricted lymphoproliferation and autoimmune phenomena, probably reflecting the fact that IL-2 also serves to constrain lymphocyte growth by enhancing lymphocyte activation-induced cell death (AICD; see below).

IL-15 has a number of effects similar to those of IL-2, primarily because it also binds to the IL-2 receptor; it is made by nonlymphoid cells, however IL-15 is essential for NK-cell development and memory T cells.

IFNγ is a major activator of macrophages; it enhances their ability to kill microorganisms, upregulates class II expression, and promotes T_H1 differentiation. Notably, IFNγ also suppresses T_H2 responses. It also acts on murine B cells to promote switching to immunoglobulin (Ig)G2a and IgG3, and inhibits switching to IgG1 and IgE. IFNγ knockout mice have diminished macrophage MHC class II expression, decreased NK function, and increased susceptibility to pathogens, especially intracellular microbes; this is also true of humans with mutations of IFNγ receptor subunits.

IL-12, as mentioned above, is a major effector molecule in T_H1 subset differentiation. It also induces proliferation, augmentation of cytolytic activity and IFNγ secretion in NK cells. IL-12 is produced by monocytes in response to pathogenic organisms; it is also produced by other cells such as B cells and dendritic cells.

IL-18 is produced by activated macrophages and is an important inducer of IFNγ. IL-18 knockout mice have impaired IFNγ production, T_H1 responses and NK cell activity.

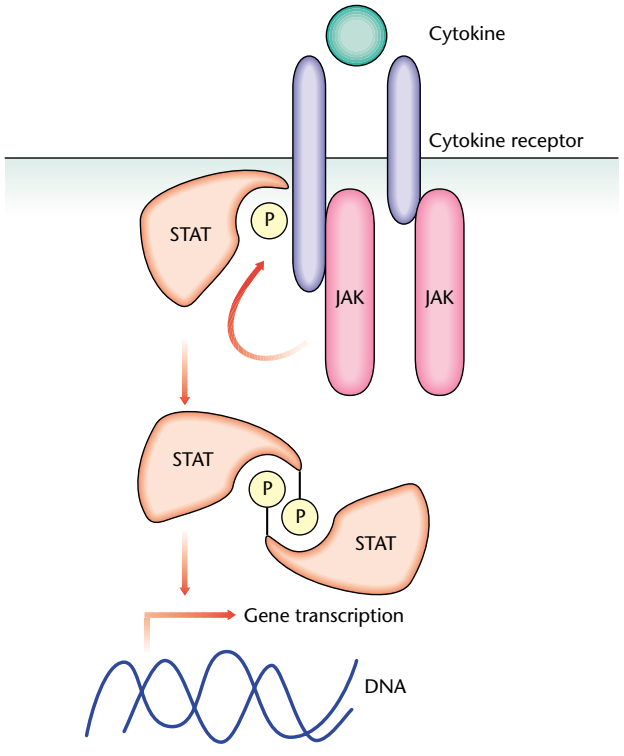

Figure 3 Signal transduction by type I and type II cytokines. Cytokines bind to the extracellular domain of transmembrane cytokine receptors, causing them to cluster. Janus kinases (JAKs) are protein tyrosine kinases that bind to the intracellular domain of cytokine receptors; the aggregation of receptors causes the JAKs to become activated. The activated JAKs phosphorylate substrates including the cytokine receptor. The phosphorylated receptor then is recognized by proteins that have specialized SH2 domains, which recognize phosphotyrosine. The signal transducer and activator of transcription (STAT) family of transcription factors have SH2 domains; they bind cytokine receptors and are themselves phosphorylated. The SH2 domains of the STATs allow them to dimerize, translocate to the nucleus, bind deoxyribonucleic acid (DNA) and regulate gene transcription.

In contrast to T_H1 cells, T_H2 cells promote responses associated with immediate hypersensitivity responses. IL-4 is made by CD4 + T_H2 cells and promotes differentiation of naive CD4 + cells to a T_H2 phenotype. It is also a growth factor for mast cells and is required for class switching of B cells to produce IgE. Importantly, IL-4 inhibits macrophage activation and blocks the effects of IFNγ. Regulation of the IL-4 gene is of great importance in dictating what kind of immune response will ensue. Consequently, a number of transcription factors contribute to its regulation. IL-13 has many of the same effects as IL-4 and even shares a receptor subunit(s) with IL-4, thus it too contributes to T_H2 responses. T_H2 cells also produce IL-5, which promotes the growth, differentiation and activation of eosinophils.

IL-10 and the TGFβ family are cytokines that antagonize lymphocyte responses. TGFβs also induce collagen and fibronectin by fibroblasts, and are thought to be responsible, in part, for diseases characterized by fibrosis such as systemic sclerosis and pulmonary fibrosis. Cells that predominantly produce IL-10 and TGFβ have been termed T_H3 (or T regulatory 1) cells.

In summary, numerous cytokines are produced in an immune response and they have important effects on the behaviour of immune cells. Some facilitate the activation of lymphocytes through effects on APCs. Other cytokines act on lymphocytes and direct them to drive cell-mediated responses, and others promote allergic responses. Still others inhibit immune responses.

Cytokine signal transduction

Cytokines that bind receptors belonging to the type I and type II cytokine receptors superfamily have several common features in their mechanisms of signal transduction. One common feature is that they activate members of a family of cytoplasmic protein tyrosine kinases known as Janus kinases (JAKs; **Figure 3** and **Table 2**), which bind cytokine receptors, become activated following cytokine binding to the receptor, and phosphorylate cytokine receptor subunits. These phosphorylated tyrosine residues recruit proteins with modules that bind phosphotyrosine (for instance, SH2 domains), one critical family of which is the signal transducers and activators of transcription (STATs) family of transcription factors. These proteins bind the phosphorylated receptor subunits, become phosphorylated themselves, dimerize and translocate to the nucleus where they regulate gene expression. Different cytokines activate different STAT proteins (**Table 2**). The specific and nonredundant functions of the different STATs are exemplified by the different STAT knockout mice (**Table 3**). STAT1-deficient mice are highly susceptible to viral infection because they lack responsiveness in interferons. In contrast, STAT4-deficient knockout mice have defective T_H1 differentiation due to abrogation of IL-12 responses, whereas STAT6-deficient mice have marked impairment in T_H2 development due to disruption of IL-4 and IL-13 signalling. These mice convincingly demonstrate the essential role of STATs in immunoregulation.

Programmed cell death or apoptosis

Upon recognition of a pathogen, immune cells are activated and produce cytokines. Typically, this response leads to the destruction and removal of the offending organism. Subsequently the immune response is turned off; indeed, a fundamental characteristic of immune responses is that they are self-limiting. A major mechanism by which this occurs is through a process termed programmed cell death, cell suicide or apoptosis. The importance of apoptosis in the developmental programmes of many species, from worms to human, is now well documented. Immune cells, though, rely heavily on these process.

Table 2 Activation of Janus kinases and signal transducers and activators of transcription by cytokines

Cytokine	JAK	STAT
Type I cytokines		
gp130-using cytokines		
IL-6, IL-11, oncostatin M, leukaemia inhibitory factor, ciliary neurotrophic factor, cardiotropin-1	JAK1, JAK2, TYK2	STAT3
IL-12[a]	JAK2, TYK2	STAT4
βc-using cytokines		
IL-3, IL-5, GM-CSF	JAK2	STAT5a, STAT5b
γc-using cytokines		
IL-2, IL-7, IL-9, IL-15	JAK1, JAK3	STAT5a, STAT5b, STAT3
IL-4[b]	JAK1, JAK3	STAT6
IL-13[b]	JAK1, JAK2, TYK2	STAT6
Hormones		
Growth hormone	JAK2	STAT5a
Prolactin	JAK2	STAT5b
Erythropoietin, thrombopoietin	JAK2	STAT5a, STAT5b
Leptin		STAT3
Type II cytokines		
IFNα/β (type I IFNs)	JAK1, TYK2	STAT1, STAT2, STAT4
IFNγ (type II IFN)	JAK1, JAK2	STAT1
IL-10[c]	JAK1, TYK2	STAT3

[a]Interleukin (IL) 12 does not use glycoprotein 130, but its receptor subunits are homologous to it.
[b]IL-4 can form two types of receptor, one with the common g subunit (gc) and one with the IL-13 receptor.
[c]IL-10 is not an interferon (IFN), but its receptor is most similar to IFN receptor subunits.
GM-CSF, granulocyte–macrophage colony-stimulating factor; gp130, glycoprotein 130; JAK, Janus kinase; STAT, signal transducer and activator of transcription; TYK2, tyrosine kinase 2.

Table 3 Characteristics of JAK and STAT knockout mice

Knockout	Pathology	Impaired signalling by
JAKs		
JAK1	Perinatal lethal, SCID	γc and gp130 cytokines, IFNs
JAK2	Embryonic lethal, defective haematopoiesis	βc cytokines, erythropoietin, thrombopoietin, IFNγ
JAK3	SCID	γc cytokines
STATs		
STAT1	Viral susceptibility	IFNα/β, IFNγ
STAT2	Not reported	
STAT3	Embryonic lethal	
STAT4	Defective T_H1 response	IL-12
STAT5a	Impaired mammary gland development	Prolactin
STAT5b	Defective sexually dimorphic growth responses	Growth hormone
STAT6	Defective T_H2 responses	IL-4, IL-13

βc, common β subunit; γc, common γ subunit; gp130, glycoprotein 130; IFN, interferon; IL, interleukin; JAK, Janus kinase; SCID, severe combined immune deficiency; STAT, signal transducer and activator of transcription; T_H, T helper cell.

There are probably a number of ways in which apoptosis is regulated by lymphocytes, but two important means are cytokine withdrawal apoptosis and AICD. That is, if the offending antigen is removed, TCR-dependent activation subsides and cytokine production falls. The needs of the expanded lymphocyte population then begin to outstrip cytokine production. Lymphocytes begin to 'starve', and this can initiate programmed cell death. Cytokine withdrawal apoptosis is also called death by neglect or passive cell death to contrast it with AICD. AICD is another important mechanism for dampening the immune response and is mediated by the interactions of the membrane molecules Fas (CD95) and Fas ligand (FasL). Fas membrane protein is expressed on lymphoid and other cells but is increased in expression with cellular activation. Its ligand, FasL, another membrane protein, is expressed on activated T cells; thus, activation of T cells results in the expression of both Fas and FasL.

The intracellular portion of the Fas receptor molecule contains 'death domains', which recruit proteins that lead to the activation of a cascade of proteases (termed caspases) which induce apoptosis. Fas–FasL interaction is a major means of mediating AICD in CD4 + cells.

Thus, it is now clear that accompanying lymphocyte activation and proliferation is substantial lymphocyte apoptosis due to either cytokine starvation or Fas–FasL-mediated caspase activation; indeed, it is quite apparent that this is a major aspect of immunoregulation. The self-limiting nature of immune responses is an essential part of immune homeostasis; programmed cell death provides an excellent mechanism for elimination of lymphocytes after the offending antigen has been cleared. The critical function of this mechanism is exemplified in two types of knockout mice. In mice lacking the IL-2Rα chain (or IL-2), there is impressive lymphoproliferation and concomitant autoimmunity, illustrating the essential role of IL-2 in constraining lymphocyte proliferation. In addition, mice with mutations of Fas or FasL also have extensive lymphoproliferative disease, as do humans with mutations of Fas (see below).

Older proposed mechanisms of immunoregulation

Suppressor cells

Lymphocytes capable of suppressing immune responses were described in the 1970s. It was initially thought that, whereas CD4 + cells were the helper subset, CD8 + cells represented the suppressor and cytotoxic subsets. However, despite numerous attempts to isolate suppressor cells, no population with exclusive suppressor activity has been found. Now we know that many cytokines are produced, some of which amplify (IL-12) and some of which suppress (TGFβ and IL-10) immune responses. Still others, like IL-4, amplify some (T_H2) and inhibit other (T_H1) responses. Thus, despite this large body of experimental data, it is tempting to conclude at this point that a unique population of suppressor cells does not exist and that most of the previously measured suppressor activity can be explained by cytokine production.

Antiidiotypic regulation

Another theory of immunoregulation was the network hypothesis of regulation. In an immune response a clone (or clones) of lymphocytes is expanded in response to a specific antigen. It was theorized that lymphocytes exist with receptors that recognize the antigen receptors on the first clonally expanded population of lymphocytes. This second population of lymphocytes was proposed to expand and control the response of the first population. This was called the antiidiotypic response. Although antiidiotypic responses can be detected experimentally, there are few data to support this mechanism as an important aspect of immunoregulation, especially for T cells.

Immunoregulation in Infectious, Allergic Autoimmune Disease and Immunodeficiency

Immune response to pathogenic organisms: protection and damage

The cellular immune response (also termed delayed-type hypersensitivity or cell-mediated immunity) is the primary defence against intracellular pathogens and can be summarized as follows. Sentinel cells, such as tissue-based macrophages and dendritic cells, engulf these pathogens and presumably migrate to the regional lymph node where they present the antigen to naive T cells. In so doing, they also produce IL-12, which aids in the development of a T_H1 response. Activated T cells produce IL-2, IFNγ, LT and chemokines. IFNγ in turn activates macrophages, enhancing their ability to kill the intracellular pathogens. T_H1 cytokines also cause the recruitment and activation of other leucocytes from the circulation.

The cellular immune response to viral infections can be summarized as follows: viral infection induces type I IFN production, which in turn inhibits viral replication and cell proliferation. It also upregulates MHC class I expression, facilitating presentation of viral peptide antigens CD8 + T cells, which become activated and lyse the cells. In addition, type I IFN activates NK cells, which also kill virally infected cells. Interestingly, viruses have evolved a number of strategies for evading the immune response including interfering with presentation of viral antigens. Fortunately, NK cells also recognize cells that lose MHC class I and kill them.

After eradication of the pathogen, the immune response generally subsides without damage to the host tissue because of the production of cytokines like TGFβ and IL-10, and because of apoptosis of lymphocytes. However, this is not necessarily the case; a normal immune response itself may cause considerable damage to the host simply because of its attempt to contain a microorganism. Cell-mediated immunity contributes to disease pathogenesis in a variety of ways. Vigorous production of TNFα can cause septic shock or lung disease, termed adult respiratory distress syndrome. Thus, sometimes patients die not from the infection but from the immune response to the infection. In addition, the cell-mediated responses to *Mycobacterium tuberculosis* infection or parasitic infestations can also extensive tissue destruction.

Role of T-cell subsets in the response to parasites

As the relationship between parasites and the host's immune system becomes elucidated better, it is becoming clear that there is a fine balance between mounting a protective immune response and one that induces pathology. In some parasitic infections, in fact, morbidity may be due to the immune or inflammatory response induced. It is apparent that both type 1 (T_H1) and type 2 (T_H2) responses can, depending on the conditions, incite tissue damage during parasitic infection.

Parasitic infections can be broadly classified into those caused by protozoa (unicellular organisms) and those by helminths (multicellular organisms). Both types of parasites have adopted numerous strategies for evading or modulating the host immune response so that chronic infections can be established. Cellular immune responses to protozoa, at least, induce a broad range of cytokines from host cells that are instrumental in controlling growth of the parasite and in restricting acute disease, although excess production can contribute quite substantially to the pathology caused by infection with these parasites. In general, however, it can be assumed that the protozoan parasites typically induce a T_H1 (or type 1 response) which, for the most part, is protective. This is certainly true for *Toxoplasma gondii* infection, the cutaneous forms of leishmanial infections, cryptosporidial infections, and probably for malaria once protection is established.

Interestingly, when there is a failure of T_H1 responses to be induced by these parasites (or when there is a loss of T_H1 cells, as occurs in the late stages of human immunodeficiency virus infection) the immunological resistance against these parasites is abrogated and growth of the parasitic protozoa goes unchecked (e.g. visceral leishmaniasis or disseminated cutaneous leishmaniasis). This has parallels in the *Mycobacterium leprae* infections in which the lepromatous form of leprosy is associated with unrestricted growth of the organism and with an inability to mount a T_H1-type response, which contrasts markedly with the less progressive forms of the infections (tuberculous leprosy) in which not only is there characteristically a T_H1-type response to the organism, but also there is a control of organism growth. Nevertheless, for some protozoal infections cytokine responses have been associated with severe morbidity, the most notable being the direct relationship between TNFα levels and the most severe form of malaria (cerebral malaria).

For the helminth infections it is less well established how cellular immune responses mediate immunity to infection or reinfection. While various immune effector mechanisms (IgE, eosinophils, mast cells) are invoked by infection with helminth, mainly mediated by the T_H2 type of cytokines, because these infections remain established within the host, often for decades, the concept of true sterile immunity to these multicellular worms remains in question. With chronic helminth infections, such as schistosomiasis, onchocerciasis and lymphatic filariasis, antigen-specific T-cell proliferation and IL-2 and IFNγ (T_H1 cytokines) are also depressed, whereas the production of IL-4 and IL-5 is consistently raised. Regulation of this T_H1 response occurs primarily through the production of counterregulatory cytokines such as IL-10 and transforming growth factor β.

This lack of T_H1 responsiveness appears to prevent parasite clearance, although murine models of human intestinal helminth infection suggest that T_H2 responses may be crucial for resistance to occur. In addition to playing a role in immunity to gastrointestinal helminths, there is strong epidemiological evidence that type 2 responses (or at least IgE) may mediate the resistance to schistosomiasis. Consistent with this general hypothesis, data from field studies in Brazil indicate that the ability to resist infection is influenced by a major gene that localizes to a region of chromosome 5 which encodes type 2 cytokines. While mediating this resistance phenomenon, it is also likely that these type 2 responses also mediate the granulomatous reactions around the schistosome eggs, responses that cause the pathological findings seen in schistosomiasis. Thus, it appears that, for all parasitic infections, the balance between proinflammatory cytokine responses and those that mediate protection is the major determinant of outcome.

Regulation of the immune response in allergic diseases and asthma

Allergic diseases are characterized by localized and/or systemic immune responses associated with immediate hypersensitivity, including IgE production, peripheral or tissue eosinophilia, and the presence of inflammatory mediator-rich mast cells and basophils. Using approaches that examine expression of either cytokines or messenger ribonucleic acid for cytokines, it has become increasingly clear that allergen-specific T cells that express IL-4, IL-5, IL-13 and IL-10 play a significant role in the pathogenesis of allergic diseases and asthma. Indeed, overproduction of the T_H2-type cytokines represents an inappropriate

response to environmental antigens, and mediates or at least prolongs allergic responses and late-phase reactions associated with airway hyperreactivity, allergic rhinitis and atopic skin disease. However, it must be mentioned that these cytokines do not always derive from T-cell sources and it may be the aggregate expression of the various cytokines *in situ* that determines the nature of the response at site of allergic inflammation. Nevertheless, regulating the overproduction of IL-4, IL-5 and IL-13 by other cytokines (IL-12, IL-18, IFNγ) or by inducing tolerance or apoptosis to the offending allergen is now undergoing evaluation.

There also appears to be a genetic predisposition to the development of allergic diatheses. Although this is likely to involve more than one gene (complex genetic trait), there is likely to be a genetic susceptibility to the production of a predominant T_H2-type cytokine response to particular allergens. Linkage to a region of chromosome 5q31.1, a region that includes genes for IL-4, IL-5 and IL-13, has been found in patients with high levels of IgE and bronchial hyperreactivity. More recently a mutation in the IL-4 receptor has been found in some patients with allergies; precisely how this contributes to disease needs to be unravelled.

Immunoregulation and the pathogenesis of autoimmune diseases

For the most part, we are rather ignorant of the pathogenesis of most autoimmune diseases. None the less, our understanding of these disease is steadily improving. It is well recognized that the development of autoimmune disease is heavily influenced by molecules such as the MHC and TCR, which are involved in antigen recognition. We also know from animal models that deficiencies of a variety of antiinflammatory cytokines like TGFβ and IL-10, or even IL-2, can lead to autoimmune disease. In addition, the overproduction of cytokines has been documented in many human autoimmune diseases as well as in animal models. For instance, patients with rheumatoid arthritis have high levels of TNFα and IL-6. Exactly why these cytokines are produced is not known. Some forms of arthritis are associated with infections, but how and why joints become damaged is still poorly understood; animal models will be key to dissecting the mechanisms involved in these diseases. The importance of apoptosis in controlling immune responses is well supported by the discovery of humans with Fas mutations; the disease is termed autoimmune lymphoproliferative syndrome. Thus, although we do not understand autoimmune and allergic diseases well, there are many clues that point to dysregulation of immune responses as a major contributor to the pathogenesis of autoimmune disease.

Immunodeficiency and immunoregulation

Immunodeficiency occurs when the key elements of the cell-mediated immune response do not function appropriately. For instance, TCR signalling is a key step in lymphocyte activation, and severe combined immune deficiency (SCID) can occur because of the absence of the TCR-associated kinase, zeta-associated protein of 70 kDa (ZAP-70).

The importance of the receptors and kinases responsible for transmitting signals from cytokine binding is underscored by two other forms of SCID. One form is due to mutation of the common γ subunit (γc), a constituent of the receptors for IL-2, IL-4, IL-7, IL-9 and IL-15. In γc deficiency, signalling via all these cytokines is disrupted, resulting in failure of the immune system to develop and function properly. The γc subunit is found on the X-chromosome and the immunodeficiency is termed XSCID. It accounts for about half of the cases of SCID. The γc subunit binds JAK3 and the γc cytokines (IL-2, IL-4, IL-7, IL-9 and IL-15) all activate this JAK. Deficiency of JAK3 also leads to a form of SCID that is identical to XSCID, except that girls and boys are both affected since the JAK3 gene resides on chromosome 19.

Recently patients have been identified with mutations of IFNγR subunits and the IL-12Rβ1 subunit. These patients become infected with pathogens that usually do not harm individuals with normally functioning immune systems, illustrating the importance of IFNγ and IL-12 in cell-mediated responses.

Most recently, patients with mutations of the TNF receptor (TNFR) have been identified and they suffer from recurrent fevers; this is apparently due to the inability to produce soluble TNFR which inhibits the inflammatory response.

Exploiting our understanding of immunoregulation to treat disease

An understanding of how the immune response is regulated allows strategies to be devised which either enhance cellular immune responses (e.g. production of better vaccines) or inhibit immune responses (e.g. prevention of transplant rejection). Some of these strategies will be discussed briefly.

Cytokines, in particular, are widely used at present. Some colony-stimulating factors (CSFs) are presently used clinically for their prohaematopoietic properties, including erythropoietin, granulocyte (G) CSF and granulocyte–macrophage (GM) CSF; it is anticipated that in the near future others will also be used. IFNα/β is used clinically in the treatment of certain infections such as chronic hepatitis B. In addition, it is used in the treatment of certain malignancies (e.g. hairy cell leukaemia) because of its antiproliferative effects. IL-2 is used in certain cancers to increase antitumour responses and is also used in patients

with acquired immune deficiency syndrome to enhance immune responses.

In contrast, anti-IL-2R antibody is used to treat transplant rejection. Similarly, interfering with TNFα action has been used therapeutically; in particular, recent studies have demonstrated the efficacy of TNF antagonists in treating rheumatoid arthritis. TNF antagonists have also been used in the treatment of septic shock. Conversely, the immunosuppressive cytokine IL-10 is now also being used experimentally in the treatment of autoimmune disease. In addition, blocking costimulatory molecules has also been used experimentally in models of autoimmune disease and transplant rejection.

Summary

In conclusion, the vertebrate immune response is the ultimate 'smart bomb'. Its ability to respond to antigen is finely tuned genetically and is modified by signals from receptors that transmit both activating and inhibitory signals. The functions of immune cells are closely controlled by secreted factors termed cytokines which can amplify, inhibit or shape the response. Finally, programmed cell death or apoptosis carefully regulates the number of circulating lymphocytes. Clearly, advances in our understanding of the molecular basis of immunoregulation have provided – and will continue to provide – important insights into the pathogenesis of immunologically mediated disease, whether they be immunodeficiencies or autoimmune diseases. Finally, these advances in basic science give us better opportunities to manipulate the immune system in a therapeutically desirable manner.

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