the cytomegaly virus, encode proteins that are functionally analogous to chemokine receptors. This allows a rapid neutralization of locally induced chemokines, and may offer an advantage to the virus. The Duffy antigen receptor for chemokines, DARC, is expressed on endothelial cells and is capable of a high-affinity binding interaction with various chemokine types. Since this receptor has no downstream signaling cascade, it is assumed to function in the presentation of chemokines to leukocytes as they flow past. DARC also functions as a receptor for *Plasmodium vivax*. CCR5 and CXCR4 are co-receptors for HIV infection of CD4+ T cells.

Antibody-Dependent Cellular Immunity and Natural Killer Cells

Lymphocytes can nonspecifically bind IgG antibodies by means of Fc receptors, then specifically attack targets cells (e.g., infected or transformed cells) using the bound antibody. This phenomenon, known as **antibody-dependent cellular cytotoxicity (ADCC)**, has been demonstrated in vitro—however its in-vivo function remains unclear. **Natural killer (NK) cells** also play a role in ADCC. The genesis of NK cells appears to be mainly thymus-independent. These cells can produce IFNγ very early following activation and do not require a specific receptor. These cells are therefore early contributors to the IFNγ-oriented TH1 immune response. NK cells can respond to cells that *do not express MHC class I* molecules, and are inactivated by contact with MHC molecules. This recognition process functions via special receptors that are not expressed in a clonal manner. NK cells probably play an important role in the *early defensive* stages of infectious diseases, although the exact nature of their role remains to be clarified (virus-induced IFNα and IFNβ promote NK activation). NK cells also appear to contribute to rejection reactions, particularly the rejection of stem cells.

Humoral, Antibody-Dependent Effector Mechanisms

The objectives of the immune response include: the inactivation (neutralization) and removal of foreign substances, microorganisms, and viruses; the rejection of exogenous cells; and the prevention of proliferation of pathologically altered cells (tumors). The systems and mechanisms involved in these effector functions are largely non-specific. Specific immune recognition by B and T cells directs these effector mechanisms to specific targets. For instance, immunoglobulins opsonize microbes (e.g., pneumococci) which are equipped with polysaccharide capsules enabling them to resist phagocyte

digestion. **Opsonization** involves the coating of such microbes with Fc-expressing antibodies which facilitates their phagocytosis by granulocytes. Many cells, particularly phagocytes (and interestingly enough also some bacteria like staphylococci), bear surface Fc receptors that interact with different Ig classes and subclasses. Mast cells and basophils bear IgE molecules, and undergo a process of degranulation following interaction with allergens against which the IgE molecules are directed. This induces the release of pharmacologically active biogenic amines (e.g., histamine). In turn, these amines represent the causative agent for physiological and clinical symptoms observed during allergic reactions (see also types I-IV, p. 108ff.).

The Complement System

The complement system (C system, Fig. 2.**16**) represents a non-specific defense system against pathogens, but can also be directed toward specific targets by antibodies. It is made up of a co-operative network of plasma proteins and cellular receptors, and is largely charged with the following tasks:

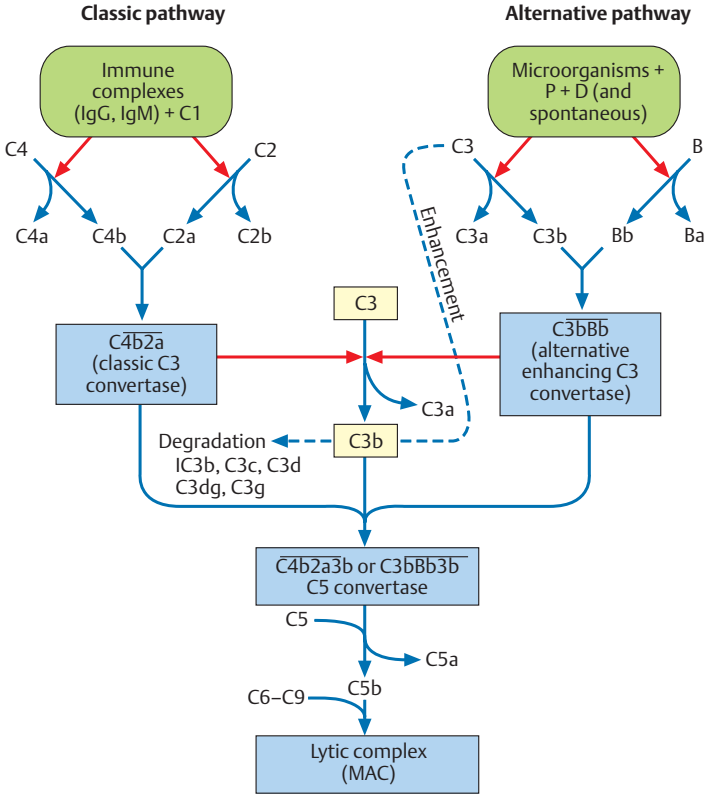
- **Opsonization** of infectious pathogens and other foreign substances, with the aim of more efficient pathogen elimination. Bound complement factors can: enhance the binding of microbes to phagocytozing cells; result in the activation of inflammatory cells; mediate chemotaxis; induce release of inflammatory mediators; direct bactericidal effects; and induce cell lysis (Fig. 2.**17**, p. 88).

Fig. 2.**16** The classic activation pathway is initiated by antigen-antibody complexes, the alternative pathway by components of microbial pathogens. The production of a C3 convertase, which splits C3 into C3a and C3b, is common to both pathways. C3b combines with C3 convertase to generate C5 convertase. C5b, produced by C5 convertase, binds to the complement factors 6–9 to form a membrane attack complex (MAC). C3b degradation products are recognized by receptors on B lymphocytes; they stimulate the production of antibodies as well as pathogen phagocytosis. The cleavage products C3a and C4a are chemotactic in their action, and stimulate expression of adhesion molecules.
Nomenclature: the components of the alternative pathway (or *cascade*) are designated by capital letters (B, D, H, I; P for properdin), those of the classical pathway (or *cascade*) plus terminal lysis are designated by "C" and an Arabic numeral (1–9). Component fragments are designated by small letters, whereby the first fragment to be split off (usually of low molecular weight) is termed "a" (e.g., C3a), the remaining (still bound) part is called "b" (e.g., C3b), the next split-off piece "c," and so on. Molecules often group to form complexes; in their designations the individual components are lined up together and are usually topped by a line. ▶

- **Solubilization** of otherwise insoluble antigen-antibody complexes.
- **Promotion of the transport** of immune complexes, and their elimination and degradation.
- **Regulation of the immune response**, achieved via their influence on antigen presentation and lymphocyte function.

Over 20 proteins of the complement system have been identified to date, and are classified as either activation or control proteins. These substances account for about 5% of the total plasma proteins (i.e., 3–4 g/l). C3 is not only present in the largest amount, but also represents a central structure for complement activation. A clear difference exists between "classic"

The Complement System: Classic and Alternative Activation



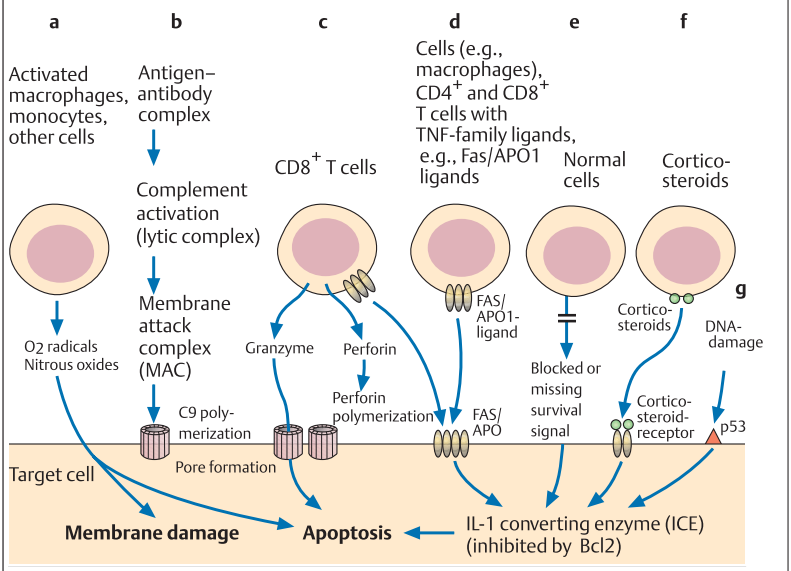


Fig. 2.**17** Oxygen radicals and nitrous oxides (**a**), MAC resulting from complement activation (**b**) and perforin (**c**) all cause membrane damage which results in cell death. Ligand binding of Fas/APO (**d**), interrupted signal receptor conduction (**e**), corticosteroid binding to receptors and intracellular structures (**f**), and DNA damage (**g**) all result in alterations of intracellular signaling cascades and lead to cellular apoptosis. (Fas = F antigen; APO = apoptosis antigen; TNF = tumor necrosis factor; Bcl2 = B-cell leukemia-2 antigen [a protein that inhibits apoptosis].)

antibody-induced complement activation and "alternative" activation via C3 (Fig. 2.**16**).

During **classic activation** of complement, C1q must be bound by at least two antigen-antibody immune complexes, to which C4 and C2 then attach themselves. Together, these three components form a C3 convertase, which then splits C3. Pentameric IgM represents a particularly efficient C activator since at least two Ig Fc components in close proximity are required for C1q binding and activation.

During **alternative activation** of complement, the splitting of C3 occurs directly via the action of products derived from microorganisms, endotoxins, polysaccharides, or aggregated IgA. C3b, which is produced in both cases, is activated by the factors B and D, then itself acts as C3 convertase. Subsequent formation of the lytic complex, C5–C9 (C5–9), is identical for both classic

and alternative activation, but is not necessarily essential since the released chemotaxins and opsonins are often alone enough to mediate the functions of microbe neutralization and elimination. Some viruses can activate the complement system without the intervention of antibodies by virtue of their ability to directly bind C1q. This appears to be largely restricted to retroviruses (including HIV). Importantly, without a stringent control mechanism complement would be activated in an uncontrolled manner, resulting in the lysis of the hosts own cells (for instance erythrocytes).

Complement Control Proteins

The following regulatory proteins of the complement system have been characterized to date:
C1 inhibitor, prevents classic complement activation.
DAF (decay accelerating factor), prevents the association of C3b with factor B, or of C4b with C2, on the cell surface. DAF can also mediate the dissolution of existing complexes, and is responsible for the regulation of classic and alternative C activities.
MCP (membrane cofactor protein), enhances the activity of the factor which degrades C3b to iC3b. Factor H and CR1 (complement receptor 1) have similar effects.
HRF (homologous restriction factor). Synonyms: MAC (membrane attack complex), inhibitory protein, C8-binding protein. HRF protects cells from C5-9-mediated lysis. This protein is lacking in patients suffering from paroxysmal nocturnal hemoglobinuria.
CD59. Synonyms: HRF20, membrane attack complex (MAC)-inhibiting factor, protectin. This is a glycolipid anchored within the cell surface which prevents C9 from binding to the C5b-8 complex, thus protecting the cell from lysis.

Those complement components with the most important biological effects include:

- **C3b**, results in the opsonization of microorganisms and other antigens, either directly or in the form of immune complexes. "C-marked" microorganisms then bind to the appropriate receptors (R) (e.g., CRI on macrophages and erythrocytes, or CR2 on B cells).
- **C3a and C5a**, contribute to the degranulation of basophils and mast cells and are therefore called anaphylatoxins. The secreted vasoactive amines (e.g., histamine) raise the level of vascular permeability, induce contraction of the smooth musculature, and stimulate arachidonic acid metabolism. C5a initiates the chemotactic recruitment of granulocytes and monocytes, promotes their aggregation, stimulates the oxidative processes, and promotes the release of the thrombocyte activating factor.
- **"Early" C factors,** in particular **C4**, interact with immune complexes and inhibit their precipitation.
- **Terminal components (C5–9),** together form the so-called membrane attack complex, MAC, which lyses microorganisms and other cells.

Some components mediate general regulatory functions on B-cell responses, especially via **CR1** and **CR2**.

Immunological Cell Death

Fig. 2.**17** summarizes the mechanisms of cell death resulting from immunological cell interactions and differentiation processes, as they are understood to date.

Immunological Tolerance

T-cell tolerance, as defined by a lack of immune reactivity can be due to a number of processes: Firstly, Negative selection in the thymus (referred to as deletion); secondly a simple lack of reactivity to antigen (self or nonself) as a result of the antigen having not been present in the secondary lymphoid organs in a sufficient quantity or for a sufficient amount of time; and thirdly an excessive stimulation of T-cells resulting from the ubiquitous presence of sufficient antigen resulting in T cell exhaustion. Finally, it may also be possible that T cells can become temporarily "anergized" by partial or incomplete antigen stimulation. As a general rule, self-reactive (autoimmune) **B cells** are not generally deleted by negative selection and can therefore be present in the periphery. Exceptions to this rule include B cells specific for membrane-bound self-determinants, some of which are deleted or anergized. B cells react promptly to antigens, even self-antigens, which are arranged repetitively. However, they only react to soluble monomeric antigens if they additionally receive T cell help. Thus, B-cell non-reactivity largely results from a lack of patterned antigen presentation structures or as a result of T-cell tolerance.

Immunological tolerance describes the concept that the immune system does not normally react to autologous structures, but maintains the ability to react against foreign antigens. Tolerance is acquired, and can be measured as the selective absence of immunological reactivity against specified antigens.

T-Cell Tolerance

A distinction can be made between **central tolerance**, which develops in the thymus and is based on the *negative selection* (deletion) of T cells recognizing self antigens present in the thymus, and **peripheral tolerance**. Peripheral tolerance results in the same outcome as central tolerance, however, this

form of tolerance involves antigen recognition by antigen-reactive peripheral T cells, followed by a process of clonal cell proliferation, end differentiation and death. The following mechanisms have been postulated, and in some cases confirmed, to account for a lack of peripheral T-cell responsiveness (Table 2.**5**, p. 71):

T-cell indifference or ignorance. Both host and foreign antigens present only within peripheral epithelial, mesenchymal or neuroectodermal cells and tissues—and which do not migrate, or are not transported by APCs, in sufficient amounts to the organized lymphoid organs—are simply ignored by T and B cells. Most self-antigens, not present in the serum or in lymphohematopoietic cells, belong to this category and are ignored despite the fact that they are potentially immunogenic. Certain viruses, and their antigens, actually take advantage of this system of ignorance. For instance, the immune system ignores the rabies virus when it is restricted to axons, and papilloma viruses as long as the antigens are restricted to keratinocytes (warts). The main reason why many self antigens, and some foreign antigens, are ignored by T cells is that immune responses can only be induced within the spleen or in lymph nodes, and non-activated (or naive) T cells do not migrate into the periphery. It has also been postulated that those naive T and B cells which do encounter antigens in the periphery will become anergized, or inactivated, due to a lack of the so-called costimulatory or secondary signals at these sites. However, the evidence supporting this theory is still indirect. Experiments seeking to understand the "indifference" of T cells are summarized in the box on p. 92f. In all probability, a great many self-antigens (as well as peripheral tumors) are ignored by the immune system in this way. These self-antigens represent a potential target for autoimmunity.

Complete, exhaustive T-cell induction. When an antigen, self or non-self, enters a lymphoid organ it encounters many APCs and T cells, resulting in the extremely efficient activation those T cells carrying the appropriate TCR. During such a scenario the responding T cells differentiate into short-lived effector cells which only survive for two to four days. This induction phase may actually correspond to the postulated phenomenon of anergy (see Table 2.**5**, p. 71). Should this be the case, anergy—defined as the inability of T cells to react to antigen stimulation in vitro—may in fact be explained by the responding cells having already entered a pathway of cell death (apoptosis) (see Fig. 2.**17**, p. 88). Once all the terminally differentiated effector T cells have died, immune reactivity against the stimulating antigen ends. Tolerance is hereafter maintained, as should the responsible antigen have entered into the thymus those newly maturing thymocytes will be subjected to the process of negative selection (e.g., as seen in chronic systemic (viremic) infections with noncytopathic viruses). Moreover, those newly matured T cells which may have escaped negative selection and emigrated into the per-

iphery will continuously be induced to undergo activation and exhaustion within the secondary lymphoid organs.

Exhaustive T-cell induction most likely occurs in responses to hepatitis C virus and HIV, and has been observed in mice experimentally infected with the noncytopathic virus causing lymphocytic choriomeningitis. Successful establishment of lymphocyte chimerism following liver transplants appears to based on the same principle. For example, a relatively short period of immunosuppression following transplantation may allow the establishment of numerous dendritic cells from the transplanted organ within the secondary lymphoid organs of the recipient, resulting in the subsequent elimination of those recipient T cells which react against the foreign MHC molecules.

Two Important Experiments addressing the induction of Immune Responses

APCs transport antigens to the peripheral lymphoid organs via the lymph vessels. *Skin flap experiment***.** To prove that antigens contacted at peripheral localizations (e.g. the skin) must first be transported on APCs *through the lymph vessels* into the local lymph node, in order to induce an immune response—an experiment was performed in which a guinea pig skin flap was prepared such that the supply vessels (lymph vessel, vein and artery) remained intact and functional.
Following sensitization of the skin flap with a contact antigen the animal reacted to a second antigenic exposure of the remaining (intact) skin with accelerated kinetics. When the lymph vessel leading from the prepared skin flap to the lymph node was interrupted, or the draining lymph node was destroyed prior to the initial sensitization, the typical secondary response was not observed—leading to the conclusion that *no T cell response was induced*. Following an initial sensitization at any other location on the skin the secondary response was observed, even on the skin flap regardless of interruption of the lymph vessel or destruction of the draining lymph node. This result indicated that the antigen-experienced effector lymphocytes reached the site of antigen via the bloodstream.
Many self antigens are ignored by $CD8^+$ cells. *A Transgenic mouse encoding a viral glycoprotein gene***.** As a comparison to the many self-antigens present in the peripheral non-lymphoid organs and cells, a gene encoding a viral glycoprotein (GP) was incorporated into mice, under the control of a regulatory gene which allowed GP expression only within the pancreatic insulin-producing β cells. This artificially integrated "self antigen" was ignored by the host's immune system, as indicated by the absence of β cell destruction or autoimmunity (diabetes). When the GP expressing transgenic mouse was infected with a virus encoding the *GP* gene, which infects lymphoid organs, GP-specific cytotoxic T cells were induced and these cells destroyed the transgenic islet cells, resulting in the onset of diabetes.
This model demonstrated that many self-antigens are ignored by the immune system simply because they are only present outside of the lymphatic system. However, should such antigens enter the immune system in a suitable form (in this case by viral infection) the host will produce an autoimmune T-cell response.

In summary, the non-responsiveness of T-cells can be achieved by: *negative selection in the thymus*; by *excessive induction in the periphery;* or by *sequestration* of the antigen outside the lymphoid organs. Persistence of the antigen within the lymphoid tissues is a prerequisite for the first two mechanisms. For the third mechanism, it is the absence of antigen within lymphatic organs which guarantees non-responsiveness. There is also a necessary role for 'second'- or 'costimulatory'-signals in the activation of T cells within lymphoid tissues, however, their role in T-cell responsiveness within solid organs remains unclear.

B-Cell Tolerance

In contrast to classic central T-cell tolerance, B cells capable of recognizing self-antigens appear *unlikely to be subjected to negative selection* (Table 2.**7**). B-cell regeneration in the bone marrow is a very intensive process, during which antigen selection probably does not play an important role. Although negative selection of bone marrow B cells can be demonstrated experimentally for highly-expressed membrane-bound MHC molecules (in antibody-transgenic mice)—this apparently does not occur for more rare membrane-bound antigens, or for most soluble self-antigens. As a general rule, these potentially self-reactive B cells are not stimulated to produce an immune response because the necessary T helper cells are not present as a result of having being subjected to negative selection in the thymus. B cell and antibody tolerance is therefore largely a result of T cell tolerance which results in the *absence of T help*.

The finding that a certain antigenic structures and sequences can activate B cells in the absence of T help indicates that autoreactive B cells which are present could be prompted to produce an IgM autoantibody response via Ig cross-linking by paracrystalline multimeric antigens. However, since self-antigens are not normally accessible to B cells in such *repetitive paracrystalline patterns*, the induction of IgM autoantibody responses is not normally observed. It is interesting to note that DNA and collagen, which often contribute to chronic autoantibody responses, exhibit repetitive antigen structures. These structures become accessible to B cells within inflamed lesions, and may therefore induce autoantibody responses in certain circumstances. A chronic autoantibody response of the IgG type, however, always requires T help arising from the presentation of self-peptides by MHC class II molecules. Ignored self-peptides, and in all likelihood infectious agents, may play a role in providing such T help. (For instance *Klebsiella* or *Yersinia* in rheumatic diseases, *Coxsackie* virus infections in diabetes, or other chronic parasitic infections.)

Table 2.7 B Cells Do Not Differentiate between Self and Nonself Antigens, but Rather Distinguish Repetitive (Usually Nonself) from Monomeric (Usually Self) Antigens

Antigen			B cells present	IgM response		
				T cell-independent	T help present	T cell-dependent
On cell membranes in the bone marrow	High concentration	Self	Unclear –	–	–	–
	Low concentration	Self	+[1]	+[1]	–	+[1]
Monomeric antigen	High concentration	Self	+[1]	not applicable	–	–
	Low concentration	Nonself	+	not applicable	+	+
Repetitive, identical 5–10 nm intervals, paracrystalline		Self (very rare)[2]	+	(+)[2]	(+)	(+)
		Nonself ("always" infectious)	+	+	+	++

[1] B cells are present and are stimulated by antigen arranged in a repetitive and paracrystalline pattern (T helper-independent type I). B-cell responses to poorly organized or monomeric antigens are not directly induced; in such cases, indirect (T helper-independent type II) or conventionally coupled T help is required.

[2] Such self antigens are not normally accessible to B cells; however collagens presents in lesions, or acetylcholine receptors, may stimulate and possibly activate B cells. When combined with T help, this activation can result in an autoimmune response.

Immunological Memory

Immunological memory is usually defined by an earlier and better immune response, mediated by increased frequencies of specific B or T cells as determined by in vitro or adoptive transfer experiments. **B-cell** immunological memory is more completely described as the ability to mediate protective immunity by means of increased antibody concentrations. Higher frequencies of specific B and T lymphocytes alone, appears to only provide limited

or no protection. Instead, immunological protection requires **antigen-dependent activation of B and T cells**, which then produce antibodies continuously or can rapidly mediate effector T functions and can rapidly migrate into peripheral tissues to control virus infections.

Usually the second time a host encounters the same antigen its immune response is both accelerated and augmented. This **secondary immune response** is certainly different from the **primary response**, however, it is still a matter of debate as to whether these parameters alone correlate with immune protection. It is not yet clear whether the difference between a primary and secondary immune response results solely from the increased numbers of antigen-specific B and T cells and their acquisition of "memory qualities", or whether immune protection is simply due to continuous antigen-induced activation (Table 2.**8**).

Table 2.**8** Characteristics of T- and B-Cell Memory

	Memory T cells		**Memory B cells**	
	Resting	Activated	Resting	Activated
Localization and migration	Blood, spleen, lymph nodes	Blood, spleen, lymph nodes, and solid tissues	Blood, spleen, lymph nodes	Germinal centers in local lymph nodes, bone marrow
Function	Secondary T-cell response	Immediate target cell lysis and interleukin release	Secondary B-cell response	Sustained IgG response
Time lapse to protective response	Slow	Fast	Slow	Immediate
Proliferation and location of proliferation	In secondary lymphoid organs	Only in secondary lymphoid organs with antigen residues	Blood, spleen	Germinal centers with antigen-IgG complexes
Antigen dependence	No	Yes	No	Yes

There is no surface marker which can unequivocally differentiate between memory T and B cells and "naive" (never before activated) cells. Instead, immunological memory is normally taken to correlate with an increased number of specific precursor T and B cells. Following an initial immunization with antigen, this increased precursor frequency of specific cells is thought to be maintained by an antigen-independent process. Yet the precursor cells can only be activated (or re-activated) by antigen, and only *activated T cells* can provide immediate protection against re-infection outside the lymphoid organs, e.g., in the solid peripheral organs. Similarly, *only antigen activated B cells can mature to become plasma cells* which maintain the increased blood antibody titers responsible for mediating protection. This indicates that residual antigen must be present to maintain protective immunological memory. As a general rule, the level of protective immunity mediated by the existence of memory T and B cells per se is minimal. Highly effective immunity and resistance to re-infection are instead provided by migratory T cells which have been recently activated (or re-activated) by antigen, and by antibody-secreting B cells. **B-cell and antibody memory** is maintained by re-encounters with antigen, or by antigen-IgG complexes which by virtue of their Fc portions or by binding to C3b are captured by-, and maintained for long periods on-, *follicular dendritic cells* present in germinal centers. **Memory T cells**, and in some cases **B cells**, can be re-stimulated and maintained in an active state by: persistent infections (e.g., tuberculosis, hepatitis B, HIV); antigen deposits in adjuvants; periodic antigen re-exposure; peptide-loaded MHC molecules with long half-lives; or possibly (but rarely) by cross-reactive antigens. Thus, secondarily activated (protective) memory T and B cells cannot easily be distinguished from primarily activated T and B cells. The antigen-dependent nature of immunological protection indeed questions the relevance of a specialized "memory quality" of B and T cells.

B-Cell Memory

It is important to differentiate between the characteristics of memory T and B cells as detected in vitro, and the salient in-vivo attributes of improved immune defenses. Following a primary immune response, increased numbers of memory B cells can of course be detected using in vitro assays or by murine experiments involving the transfer of cells into naive recipients. However, these increased B cell frequencies do not necessarily ensure immune protection against, for instance, viral re-infection. Such protection requires the existence of an increased titer of protective antibodies within the host.

Why is Immunological Memory Necessary?

A host which does not survive an initial infection obviously does not require further immunological memory. On the other hand, survival of the initial infection proves that the host's immune system can control or defeat the infection, once again apparently negating the need for immunological memory. Even assuming that better immune defenses provide a clear evolutionary advantage, especially during pregnancy, the idea of immunological memory must be understood as protection within a developmental framework:

1. Due to MHC restriction of T-cell recognition, it is not possible for a mother to pass on T-cell immunological experience to her progeny as the histoincompatibility reaction would induce mutual cellular rejection. For the same reason, a child's T cells apparently cannot mature until relatively late in its development (usually around the time of birth). This explains why newborns are almost entirely lacking in active immune defenses (Fig. 2.**18**). Newborn mice require about three to four weeks (humans three to nine months) before the T-cell immune response and the process of T-B cell collaboration which results in the generation of antibody responses become fully functional. During this period passive immune protection is essential. This type of protection is mediated by the transfer of protective, largely IgG, antibodies from mother to child through the placenta during pregnancy, and to some extent within the mother's milk. An example of this is provided by cattle where the acquisition of colostral milk by the calf is essential to its survival. Calves can only access protective IgG through the colostral milk delivered during the first 24 hours after birth (fetal calf serum contains no Ig). During the first 18 hours post partum, the calf's intestine expresses Fc receptors which allow the uptake of undigested antibodies from the mothers milk into the bloodstream. How can comprehensive, transferable, antibody-mediated protection be ensured under these conditions? During a three-week murine or 270-day human pregnancy, mothers do not normally undergo all of the major types of infection (indeed infection can be potentially life-threatening for both the embryo/fetus and the mother), and so the array of antibodies required for comprehensive protection cannot be accumulated during this period alone. Instead, an accumulation of the immunological protective antibody levels representing the **immunological life experience of infections in the mother's serum** is necessary. The female sex hormones also encourage Ig synthesis, correlating with women's higher risk level (about fivefold) for developing autoantibody diseases (e.g., lupus), and for autoimmune diseases in general.

2. Reproduction requires a relatively **good level of health** and a good nutritional status **of the mother**. However, it also requires an effective immune defense status within the population (herd), including males, since all would otherwise be threatened by repeated and severe infections. The increased frequency of specific precursor B and T cells improves immune defenses against such infections. However, this relative protection is in clear contrast to the absolute protection an immunoincompetent newborn requires to survive.

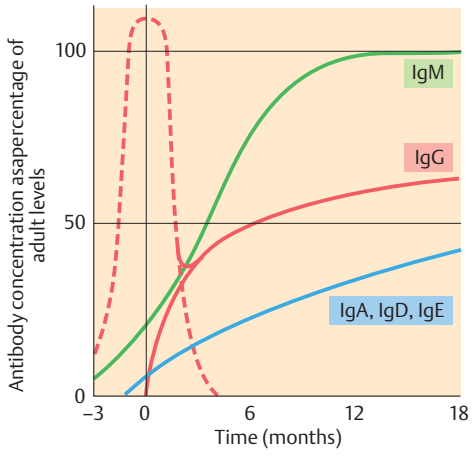


Fig. 2.**18** Synthesis of significant amounts of immunoglobulins only begins during the perinatal period (uninterrupted lines). IgG from the mother is therefore the child's main means of protective immunity before the age of three to six months (dotted line). Infections encountered during this early period are attenuated by maternal antibodies, rendering such infections vaccine-like.

T-Cell Memory

As with B cells and antibodies, enhanced defenses against intracellular pathogens (especially viruses and intracellular bacteria) does not solely depend on increased numbers of specific T cells, but rather is determined by the *activation status* of T cells. Here again it must be emphasized that protective immunological memory against most bacteria, bacterial toxins, and viruses, is mediated by *antibodies*! *Memory T cells* are nonetheless important in the *control of intracellular bacterial infections* (e.g., tuberculosis [TB], leprosy), as well as persistent *noncytopathic viruses* such as hepatitis B and HIV (see also p. 106). It has been demonstrated, at least in mouse models, that a higher number of T cells alone is often insufficient for the protection of the host against the immunopathological consequences of a defensive $CD8^+$ T-cell response. Yet such T cell responses must be activated in order to provide immunity. In the case of *tuberculosis*, sustained activation of a controlled T-cell response by minimal infection foci was postulated, and confirmed, in the 1960s as constituting **infection immunity—**i.e. the lifelong, and usually effective, immune control of the disease by an ongoing localized low-level of infection. A similar situation is observed for cell-mediated immune responses against leprosy, salmonellae, and numerous parasitic diseases (often together with antibodies). The existence of infection-immunity explains why apparently controlled, minimal, infections tend to *flare up* when the immune system is *compromised* by cytostatic drugs, age, or HIV infection. Delayed type

(dermal) hypersensitivity (DTH, see below and p. 114f.) can be applied diagnostically to determine infection immunity (for example against tuberculosis and leprosy), since the existence of continued infection continuously activates those T cells required for both pathogen control and DTH reactions.

Delayed Dermal Hypersensitivity Reaction

The classic example of a **delayed type hypersensitivity (DTH)** reaction is the **tuberculin reaction** (Mantoux test in humans). It was one of the first specific cell-mediated immune responses to be identified—as early as the 1940s in guinea pigs. The response is specific for MHC class II antigens and is $CD4^+$ T cell-dependent. In some cases, especially during active viral infections, a DTH reaction is transiently observed and is mediated by $CD8^+$ T cells. The simplest way to elicit a DTH reaction is to introduce a diagnostic protein, obtained from the pathogen, into the skin. The test reaction will only develop should continuously activated T cells be present within the host, since only these cells are capable of migrating to dermal locations within 24–48 hours. If no activated T cells are present, re-activation within the local lymph nodes must first take place, and hence migration into the dermis will require more time. By this time the small amount of introduced diagnostic peptide, or protein, will have been digested or will have decayed and thus will no longer be present at the injection site in the quantity required for induction of a local reaction.
A positive delayed hypersensitivity reaction is, therefore, an indicator of the presence of activated T cells. The absence of a reaction indicates either that the host had never been in contact with the antigen, or that the host no longer possesses activated T cells. In the case of tuberculosis, a negative skin test can indicate that; no more antigen or granuloma tissue is present, or that the systemic immune response is massive and the pathogen is spread throughout the body. In the latter case, the amount of diagnostic protein used is normally insufficient for the attraction of responsive T cells to the site of injection, and as a consequence no measurable reaction becomes evident (so that the Mantoux test may be negative in Landouzy sepsis or miliary tuberculosis). DTH reactions provide a diagnostic test for **tuberculosis** (Mantoux test), **leprosy** (lepromin test), and **Boeck's sarcoid** (Kveim test). However, these dermal reactions may disappear in those patients that are immunosuppressed or infected with measles or AIDS.

Immune Defenses against Infection and Tumor Immunity

Protection against infections can be mediated by either; non-specific defense mechanisms (interferons, NK cells), or specific immunity in the form of antibodies and T cells which release cytokines and mediate contact- and perforin-dependent cell lysis. Control of cytopathic viruses requires soluble factors (antibodies, cytokines), whilst control of noncytopathic viruses

and tumors is more likely to be mediated via perforins and cytolysis. However, cytotoxic immune responses can also cause disease, especially during noncytopathic infections. Development of an evolutionary balance between infectious agents and immune responses is an ongoing process, as reflected by the numerous mechanisms employed by pathogens and tumors to evade immune-mediated defenses.

All immune defense mechanisms (see Fig. 2.**1**, p. 44) are important in the battle against infections. Natural humoral mechanisms (antibodies, complement, and cytokines) and cellular mechanisms (phagocytes, natural killer cells, T cells) are deployed by the immune system in different relative amounts, during different phases of infection, and in varying combinations. Gross simplifications are not very helpful in the immunological field, but a small number of tenable rules can be defined based on certain model infections. Such models are mainly based on experiments carried out in mice, or on clinical experience with immunodeficient patients (Fig. 2.**19**).

General Rules Applying to Infection Defenses

- **Non-specific defenses** are very important (e.g., Toll-like receptors, IFNα/β), and 'natural immunity' (meaning not intentionally or specifically induced) represented by natural antibodies, direct complement activation, NK cell and phagocytes, plays a significant role in all infections. However, much remains to be learned about their roles.
- **Antibodies** represent potent effector molecules against acute bacterial infections, bacterial toxins, viral re-infections, and in many cases against acute cytopathic primary viral infections (e.g., rabies and influenza). Antibodies are also likely to make a major contribution to the host-parasite balance occurring during chronic parasitic infections. IgA is the most important defense mechanism at mucosal surfaces (Fig. 2.**5**, p. 57).
- **Perforin-dependent cytotoxicity in $CD8^+$ T cells** is important for defense against noncytopathic viruses, for the release of chronic intracellular bacteria, and for protection against intracellular stages of certain parasites.
- **Nonlytic T-cell responses** provide protection in the form of cytokines (very important cytokines include IFNγ and TNFα), which promote the enhanced digestion and destruction of intracellular bacteria and parasites (e.g., listeria, leishmania, etc.), and in some situations enhance immunity against complex viruses (e.g., the smallpox virus) (Fig. 2.**15**, p. 79). Infectious agents apparently induce cytokines within a matter of hours (for instance IFNγ, IL-12 , and IL-4), and this early cytokine production in turn functions to define the ensuing T cell response as type 1 or type 2 (see p. 75 and Fig. 2.**14**, p. 78).

General Schemes of Infectious Diseases

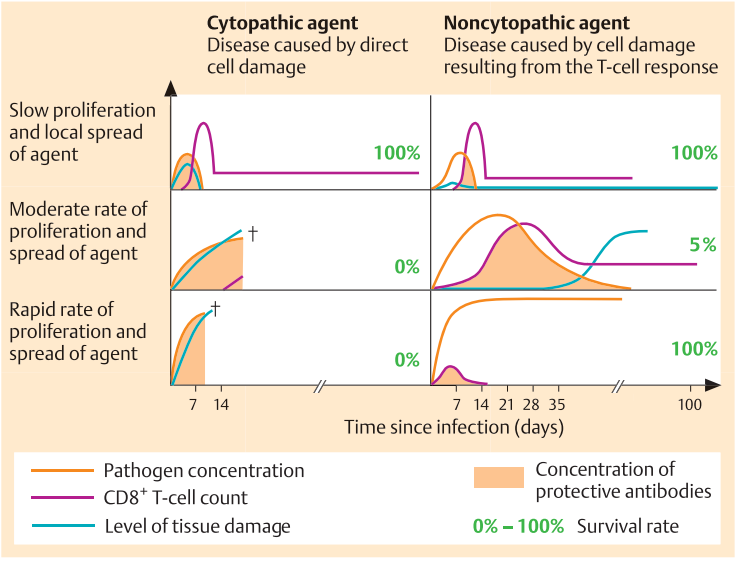


Fig. 2.**19** The degree of host survival depends on both the rate of proliferation, and the extent of spread, of an infectious agent – as well as the intensity of the host's cytotoxic T-cell response. Infection by cytopathic pathogens can only be controlled if pathogenic proliferation is slow and the pathogen remains localized; otherwise the outcome is usually fatal. In the case of noncytopathic pathogens, the cytotoxic T-cell response is the critical parameter. Pathogens which proliferate slowly are quickly eradicated. The T-cell response can be halted by pathogens which proliferate rapidly and spread widely due to the deletion of responding T cells. The degree of survival for hosts is high in both of these cases. For pathogens which exhibit moderate rates of proliferation and spread, the T-cell response may cause extensive immunopathological damage, and thus reduce the proportion of surviving hosts, some of which will controll virus, some not.
A weakened immune defense system may not progress beyond an unfavorable virus-host balance, even when confronted with a static or slowly replicating pathogen which represents an initially favorable balance.

IgE-mediated defense is important, along with IgA, in enhancing the elimination of gastrointestinal, pulmonary, and dermal parasites. Although details of the process are still sketchy, IgE-dependent basophil and eosinophil defense mechanisms have been described for model schistosomal infections.

Avoidance strategies. Infectious agents have developed a variety of strategies by which they can sometimes succeed in circumventing or escaping immune responses, often by inhibiting cytokine action.

Antibacterial Immune Effector Mechanisms

Extracellular bacteria. Capsules with carbohydrate elements render bacteria more resistant to efficient phagocytosis and digestion (mainly by granulocytes)—however, highly repetitive carbohydrate surface antigens induce efficient B cells responses which do not require T help and which are supported in part by lipopolysaccharides (LPS). *Pure carbohydrates do not induce T help!* Short-lived IgM responses can control bacteria in the blood effectively, but are usually insufficient in the control of toxins. In such cases, immunoglobulins of the IgG class are more efficient, as a result of their longer half-life and greater facility for diffusing into tissues.

Intracellular bacteria are controlled by T cells (mainly via T cell secreted IFNγ and TNFα which activate macrophages), or in some cases by the release of intracellular bacteria through $CD8^+$ T cell mediated cellular destruction.

Avoidance Mechanisms of Pathogens (with examples)

Influence on the complement system. Some pathogens prevent complement factors from binding to their surfaces:
- Prevention of C4b binding; herpes virus, smallpox virus.
- Prevention of C3b binding; herpes simplex virus (imitates DAF, see p. 86), trypanosomes.

Compartmentalization in non-lymphoid organs. Viruses can avoid confrontation with the immune defenses by restricting their location to peripheral cells and organs located outside of lymphoid tissues:
- Papilloma viruses; infect keratinocytes.
- Rabies virus; infects neurons.

Modulation and down-regulation of surface antigens. Infection agents can avoid immune defenses by mutating or reducing their expression of T- or B-cell epitopes.
- Influenza viruses; antigenic shift caused by rearrangement of genetic elements or drift resulting from mutation of hemagglutinin (at the population level).
- Gonococci; recombination of pili genes.
- Schistosoma; mutation of envelope proteins or masking by adoption of host MHC antigens.

Interference with phagocytosis and digestion. *Mycobacterium tuberculosis* uses CR1, CR2, or fibronectin as a receptor for cell entry; it does not induce efficient oxidative mechanisms in macrophages.
- Components of bacterial cell walls can impede phagosome-lysosome fusion and are resistant to digestion.
- Heat shock proteins (hsp60 and hsp70) or superoxidedismutase aid resistance.

Continued: **Avoidance Mechanisms of Pathogens (with examples)**

Influence on lymphocytes and immunosuppression.
- Direct destruction of lymphocytes, or negative regulation of their function (HIV?).
- Induction of immunopathological T-cell responses (in some cases these can be immunosuppressive, e.g. HIV).
- Induction of immunosuppressive autoantibodies.

Influence on selection, induction, and deletion of T cells.
- Negative selection of T cells; if viral antigens are present in the thymus responsive T cells will be deleted.
- Exhaustive activation, and subsequent deletion, of peripheral T cells; in some overwhelming peripheral virus infections all of the responding T cells are deleted (HBV, HCV).

Interference with cytokines, cytokine and chemotaxin receptors (R), etc. Many viruses produce substances that block or inhibit receptors for the humoral components of the immune defense system, for instance:
- IL-1βR, TNFαR, IFNγR; herpesvirus, smallpox virus.
- Chemotaxin receptor; cytomegalovirus.
- IL-10R; the Epstein-Barr virus produces B-cell receptor factor I, which binds to the IL-10R thus preventing activation of TH2 cells.
- Viral-induced inhibition of interleukin production.

Impairment of MHC antigen expression. Down-regulation of MHC class I and/or class II expression:
- Adenovirus; E19 protein reduces expression of MHC class I on infected cells.
- Murine cytomegalovirus; prevents transport of MHC class I to the Golgi apparatus.

Immune Protection and Immunopathology

Whether the consequences of an immune response are protective or harmful depends on the balance between infectious spread and the strength of the ensuing immune response. As for most biological systems, the immune defense system is optimized to succeed in 50–90% of cases, not for 100% of cases. For example, immune destruction of virus-infested host cells during the eclipse phase of a virus infection represents a potent means of preventing virus replication (Fig. 2.**15**, p. 79). From this point of view, **lytic CD8⁺ T-cell responses** make good sense as the host will die if proliferation of a *cytopathic virus* is not halted early on. If a *noncytopathic virus* is not brought under immediate control, the primary illness is not severe—however, the delayed cytotoxic response may then lead to the destruction of very large numbers of infected host cells and thus exacerbate disease (Tables 2.**9** and 2.**10**). Since an infection with noncytopathic viruses is not in itself life-threatening to the

Table 2.9 Balance between Infection and Host Immunity: Effect on the Disease

Infectious agent	Cytopathogenicity of agent	Efficiency of immune response		
		Early start	*Later start*	*No immune response*
Extracellular bacteria				
Meningococci Staphylococci	High	Recovery	Death	Death
Facultatively intracellular bacteria				
Listeria	High	Recovery	Death	Death
Tuberculosis bacilli	Moderate	Recovery	Immunopathological inflammation	Miliary tuberculosis (early death) Landouzy sepsis (very early death)
Leprosy bacilli	Very low	Recovery	Tuberculoid leprosy	Lepromatous leprosy (late death)
Viruses				
Smallpox virus	High	Recovery	Death	Death (early)
LCMV (lymphocytic choriomeningitis)	Very low	Recovery	Immunopathological disease	Healthy carrier
Hepatitis B virus	Very low	Recovery	Aggressive hepatitis	Carrier (very late liver carcinoma)
HIV	Low (?)	Recovery	AIDS	Healthy carrier (occult infection) (?)
Unrecognized and unknown infections, viruses, bacteria, and endogenous retroviruses	Low	?	Autoimmunity	"Healthy" or occult carrier (although infectious agent is unknown)
Clinical symptoms		None	Chronic disease	Variable disease symptoms, sometimes delayed or asymptomatic

Table 2.**10** Hepatitis B Virus (HBV) Infection. Inter-relations between Efficient Antigen Presentation by MHC Molecules, T-Cell Responses, Course of Infection, and Clinical Picture. Decreased Immunocompetence or Enhanced HBV Proliferation Shifts the Balance Towards an Unfavorable Outcome; Vaccination Shifts the Balance Towards a Favorable Outcome

Presentation of HBV antigen by MHC	T-cell response	Kinetics of infection	Clinical phenotype
+++	*Early*	HBV proliferation is halted	Acute hepatitis with or without icterus, due to hepatocyte damage being minimal
+/-	*Late*	HBV proliferation is halted too late. Liver cells are lysed by $CD8^+$ T cells	Acute to chronic aggressive hepatitis
–	*None*	HBV proliferation is not halted, but there is no immunopathology	Healthy HBV carrier (late liver cell carcinoma)

host, it is paradoxically the immune response that is responsible for pathology and illness due to its ability to destroy infected host tissue.

Hepatitis B viral infections in humans (Table 2.**10**), and LCMV infections (lymphocytic choriomeningitis) in mice, are amongst the most thoroughly studied examples of this potentially negative consequence of protective immune responses. A similar situation is also observed for the cellular immune response against facultative intracellular tuberculosis and leprosy bacilli which themselves have relatively low levels of pathogenicity (Table 2.**9**). A healthy immune system will normally bring such infectious agents under control efficiently, and the immunological cell and tissue damage (which occurs in parallel with the elimination of the pathogen) will be minimal, ensuring that there is little by way of pathological or clinical consequence. However, should the immune system allow these agents to spread further, the result will be a chronic immunopathological response and resultant tissue destruction—as seen during hepatitis B as *chronic or acute aggressive hepatitis* and in leprosy as the *tuberculoid form*. Should a rapidly spreading infection result in exhaustion of the T cell response, or should an insufficient level of immunity be generated, the infected host will become a carrier. This carrier state, which only occurs during infections characterized by an absent or low-level of cytopathology, is convincingly demonstrated in hepatitis B carriers and sufferers of lepromatous leprosy.

Immunopathological Damage and AIDS

Could it be that immunopathological damage resulting from T cell immune responses play a role in AIDS?

The general assumption at the present time is that the causal HIV virus destroys those T helper cells it infects, yet no unequivocal in-vivo proof of this assumption has been obtained. T helper cells do disappear, but how and why they disappear remains unclear. Animal models employing viruses similar to HIV suggest that AIDS might also develop by alternative means:

Assuming that HIV is a *noncytopathic, or only mildly cytopathic*, virus—infection of macrophages, dendritic cells, and/or T helper cells will not cause an immediate outbreak of disease. Soon, however, the virus-infected macrophages and T helper cells will be destroyed by specifically reacting cytotoxic $CD8^+$ T cells. Because the immune response also acts to inhibit virus proliferation, the process of cellular destruction is generally a gradual process. However, over time the immune system itself may become damaged and weakened. Paradoxically, the process of immunological cell destruction would help the virus survive for longer periods in the host and hence facilitate its transmission. From the point of view of the virus this would be an astounding, and highly advantageous, strategy—but one with tragic consequences for the host following, in most cases, a lengthy illness. If proliferation of HIV could be slowed or even halted, the virus would infect fewer lymphocytes, and thus fewer cells would be destroyed by the cytotoxic T-cell response. Prevention or reduction of HIV proliferation, either by pharmacological means or by bolstering the early immune defenses through other means, therefore represents an important objective despite the likelihood that HIV is not very cytopathic.

Influence of Prophylactic Immunization on the Immune Defenses

Vaccines provide protection from diseases, but in most cases cannot entirely prevent re-infection. Vaccination normally results in a limited infection by an attenuated pathogen, or induces immunity through the use of killed pathogens or toxoids. The former type of vaccine produces a very *mild infection or illness* capable of inducing an immune response and which subsequently protects the host against re-infection. The successful eradication of smallpox in the seventies so far represents the greatest success story in the history of vaccination. The fact is that vaccinations never offer absolute security, but instead improve the chances of survival by a factor of 100 to 10 000. A special situation applies to infections with noncytopathic agents in which disease results from the immune response itself (see above). Under certain circumstances, and in a small number of vaccinated persons, the vaccination procedure may therefore shift the balance between immune defense and infection towards an unfavorable outcome, such that the vaccination will actually *strengthen the disease*. Rare examples of this phenomenon may include the

use of inactivated vaccines against the respiratory syncytial virus (RSV) in the sixties, and experience with certain so-called subunit vaccines and recombinant vaccines against noncytopathic viral infections in rare model situations. Generally, it should be kept in mind that most of the successful immunization programs developed to date have mediated *protection via antibodies*. This particularly applies to the classic protective vaccines listed in Table 1.**13** (p. 33) for children, and explains why antibodies not only are responsible for the protection of neonates during the immuno-incompetent early postnatal period where immunological experience is passed on from the mother via antibodies, but also attenuate early childhood infections to become vaccine-like. This explains why successful vaccines all protect via neutralizing antibodies, because this pathway has been selected by co-evolution. As mentioned earlier, with regard to immunological memory, memory T cells appear to be essential to host immune protection, particularly in those situations when antigen persistence is controlled efficiently by means of infection-immunity (e.g., tuberculosis, HIV).

Tumor Immunity

Our knowledge concerning the immune control of tumors is still modest. Some tumor types bear defined tumor-associated, or tumor-specific, antigens. However this is apparently not sufficient for induction of an efficient immune defense. There is also the problem of tumor diagnosis; the presence of tumors is sometimes confirmed using a functional or immunological basis, yet the tumor cannot be located because conventional examinations are often unable to discover them until they reach a size of about 10^9 cells (i.e., about 1 ml) of tumor tissue.

Factors important in immune defense reactions include the location and rate of proliferation, vascularization or the lack thereof, and necrosis with phagocytosis of disintegrating tumor tissue. We never actually get to see those rare tumors against which immune control might have been successfully elicited, instead we only see those clinically relevant tumors that have unfortunately become successful tumors which have escaped immune control.

Evidence of the immune system's role in tumor control includes:

- Greater than 85% of all tumors are carcinomas and sarcomas, that is nonlymphohematopoietic tumors which arise in the periphery, outside of organized lymphoid tissues. The immune system, in a manner similar to that seen for many strictly extra-lymphatic self antigens, ignores such tumors at first.

- Lymphohematopoietic tumors often present immunological oddities such as unusually low, or entirely absent, MHC and/or low tumor antigen concentrations, plus they frequently lack accessory molecules and signals.

- Congenital or acquired immunodeficiency—whether caused by anti-lymphocytic sera, cytostatic drugs, gamma irradiation, UV irradiation, or infection—usually encourages tumor growth, especially for lymphohematopoietic tumors. Carcinomas and sarcomas show little or no increased susceptibility. Interestingly, experimental carcinogens are frequently also immunosuppressive.
- Surgical removal of a large primary tumor may result in the disappearance (or rarely in rapid growth) of metastases within the lymph nodes.
- Tumor cells often display modulated MHC expression—some tumors lack MHC class I molecules entirely—or in some cases tumors selectively down-modulate the only MHC allele capable of presenting a specific tumor-associated peptide (e.g., the colon adenocarcinomas). Other tumors side-step immune defenses by down-regulating tumor-specific antigens.
- The immune response may fail if tumor differentiation antigens are expressed, against which the host exhibits an immunological tolerance (e.g., carcinoembryonic antigen [CEA], T-cell leukemia antigen).
- Blockade of the reticuloendothelial system may encourage the development of lymphohematopoietic tumors. For instance, chronic parasitic infections or infection by malaria can result in the development of Burkitt lymphoma, a B-cell malignancy.

The Pathological Immune Response

An immune response can also cause disease. Such responses can be classified into the following types: **Type I:** allergic IgE-dependent diseases; **Type II:** antibody-dependent responses to cell membranes, blood group antigens or other auto-antigens; **Type III:** immune complex-initiated diseases whereby surplus antigen-antibody complexes are deposited on basement membranes, resulting in development of chronic disease via complement activation and inflammatory reactions; **Type IV:** cellular immunopathology resulting from excessive T-cell responses against infections that otherwise exhibit low cytopathogenicity, or against allogenic organ transplants.

Type I: IgE-Triggered Anaphylaxis

This type of immediate hypersensitivity reaction occurs within minutes in allergically sensitized individuals. Although serum IgE has a short half-life (one to two days), IgE antibodies bound to the Fc_{ε} receptor on basophils

and mast cells have a half-life of several months and when bound by the specific allergen mediate cellular degranulation and the release of biogenic amines (e.g., histamine, serotonin). These mediators can influence the smooth musculature, and mainly result in the constriction of the pulmonary- and broncho-postcapillary venules, together with arteriole dilation. The local manifestations of IgE-triggered anaphylaxis include whealing of the skin (urticaria), diarrhea for food allergies, rhinitis or asthma for pollen allergies, or a generalized anaphylactic shock. IgE reactions are usually measured in vitro using RIA (radioimmunoassay), RIST (radioimmunosorbent test) or RAST (radioallergosorbent test) (see Fig. 2.**28** and Fig. 2.**29**, p. 131f.) Frequent causal agents of IgE allergies in humans include pollen, animal hair, house dust (mites), insect bites and stings, penicillin, and foods. Examples of allergic diseases include local allergic rhinitis and conjunctivitis, allergic bronchial asthma, systemic anaphylactic shock, insect toxin allergies, house dust (mite) and food allergies, urticaria, and angioedemas.

Degranulation of mast cells and basophils can be induced by factors other than the cross-linking of specific IgE antibodies. Such factors include the complement factors C3a and C5a, and pharmacological inducers ("pseudo-allergy!").

Atopic patients suffer severely from allergies. Atopia is genetically conditioned, with a child exhibiting a 50% risk of developing atopy if both parents are allergic, or a 30% risk if only one parent is allergic. The incidence level of atopy within the general population is roughly 10–15%. Atopia correlates with high levels of IgE production, and desensitization refers to attempts to change a TH2 (IgE-producing) response into a TH1 (IgG-favoring) response by means of repeated inoculations or oral doses of allergens (see Fig. 2.**14**, p. 78). It is likely that increased production of IgG—as opposed to IgE—antibodies plays a major role in the success of desensitization. IgE no doubt has an important biological function, probably against ectoparasites, with allergic reactions representing nothing more than an unfortunate side effect of this biological system. Little research has been performed on the nature of the protective function of IgE during parasitic infections (or on the role of eosinophils). However, we do know that mediators released by IgE-triggering of mast cells and basophils cause the smooth intestinal musculature to contract, and in this way facilitate the elimination of intestinal parasites.

Type II: Cytotoxic Humoral Immune Responses

These are pathological immune responses induced by the binding of IgM or IgG antibodies to antigens present on a cell surface (including viral products or haptens), or within tissue components. The mediators responsible for such tissue damage are usually components of the complement system,

Table 2.**11** Examples of Antibody–Related Type II Immunopathologies

Antibody	Autoimmune pathology or immunopathology
Anti-cell membrane	– Rhesus incompatibility – Blood transfusion complications – Autoimmune hemolytic anemia – Immune neutropenia, idiopathic thrombocytopenia
Anti-basement membrane	– Goodpasture syndrome
Anti-collagen	– Sclerodermia – Pemphigoid (anti-epidermal basal membrane)
Anti-desmosome	– Pemphigus vulgaris
Anti-receptor	– Anti-acetylcholine receptors: myasthenia gravis – Anti-TSH receptors: Basedow disease
Anti-hormone	– Anti-thyroid hormone (Hashimoto thyroiditis) – Anti-intrinsic factor (pernicious anemia)
Anti-medication	– Chemical groups (haptens) bound to cell surface (cytolysis, agranulocytosis)
Anti-cell component	– Anti-DNA (lupus erythematosus, LE) – Anti-mitochondrial (LE, Hashimoto thyroiditis)

or granulocytic digestive enzymes. The most important diseases resulting from cytotoxic humoral immune responses are listed in Table 2.**11**.

Autoantibody Responses

Some clinically important autoantibodies are directed against hormone receptors, for example thyrotoxicosis in Basedow's disease is caused by autoantibodies that stimulate the TSH receptor, and myasthenia gravis is caused by blockage of the acetylcholine receptor by specific autoantibodies. Other antibody-induced diseases mediated by antibodies, directed against hormones and other cellular self antigens, include Hashimoto thyroiditis (induced by anti-thyroglobulin and anti-mitochondrial autoantibodies), pernicious anemia (anti-intrinsic factor), pemphigus vulgaris (anti-desmosome) Guillain-Barré syndrome (ascending paralysis caused by specific myelin autoantibodies), and scleroderma (involving anti-collagen antibodies). Other immunopathologies involving autoantibodies include transplant rejection as a result of endothelial damage (especially in xenogeneic transplants), and tumor rejection caused by antibodies against tumor-associated antigens present on neoplastic cells (especially relevant for lymphohematopoietic

Table 2.**12** Mechanisms Of Autoantibody Induction

Possible mechanisms	Autoimmune pathology or immunopathology
Polyclonal B-cell activation	Lipopolysaccharides, viruses, chronic parasitic infection
Molecular mimicry (overall very rare)	Anti-tat (HTLV-1), anti-H. pylori, or anti-streptococcus crossreacting with self-antigens
Exposure of hidden autoantigens	Cytopathic effects of infectious agents
Adjuvant effects	In the presence of granuloma formation and chronic inflammatory reactions lymphoid tissue may form in peripheral organs (e.g., during Hashimoto's thyroiditis)
Breakdown of tolerance	Due to coupling of T helper epitopes to autoantigens, possible in connection with virus infections of cells

tumors). However, in general the detection of autoantibodies does not necessarily correlate with evidence of pathological changes or processes. In fact, our detection methods often measure low-avidity autoantibodies that may have no direct disease-causing effects.

Exactly how autoantibody responses are induced remains to be clarified. As explained earlier (in the discussion of immunological tolerance) such IgG responses cannot be induced without T help. Thus, intensive research is currently focused on those mechanisms by which T cell help for autoreactive B cells is regulated; Table 2.**12** sums up some of the possible mechanisms.

Anti-blood Group Antibody Reactions

ABO system. These B-cell epitopes consist of *sugar groups present in the membranes of red blood cells*. The four classic blood groups are determined by one gene with three alleles. This gene controls glycosylation. The O allele codes only for a basic cell surface structure (H substance) with the terminal sugars galactose and fucose. The A allele adds *N*-acetylgalactosamine to this basic structure, the B allele adds galactose. This results in epitopes, which are also seen frequently in nature largely as *components of intestinal bacteria*. Individuals who carry the A allele are tolerant to the A-coded epitope, whilst individuals with the B allele are tolerant to the B epitope. Individuals who carry both of these alleles (genotype AB) are tolerant to both epitopes, whereas persons who are homozygotes for the O allele are not tolerant to either A or B. Following birth, the intestinal tract is colonized by bacteria con-

taining large numbers of epitopes similar to the A and B epitopes. During the first months of life, people with blood group O (homozygous for the O allele) produce both anti-A and anti-B antibodies, people with blood group A (genotype AO or AA) produce only anti-B antibodies, people with blood group B (genotype BO or BB) produce only anti-A antibodies, and people with blood group AB produce neither anti-A or anti-B antibodies.

These so-called "natural" antibodies (meaning these antibodies are produced without a recognizable immunization process) are of the IgM class; there is usually no switch to IgG, probably resulting from a lack of necessary helper T-cell epitopes. The presence of the blood group antibodies makes blood transfusions between non-matched individuals extremely risky, necessitating that the blood group of both the donor and recipient is determined before the blood transfusion takes place. Nevertheless, the *antibodies in the donor blood are not so important* because they are diluted. The O genotype is therefore a universal donor. Note that IgM antibodies to blood groups present no danger to the fetus since they cannot pass through the placental barrier.

Rhesus factor. This system is also based on genetically determined antigens present on red blood cells, although as a general rule there is no production of "natural" antibodies against these. IgM and IgG antibodies are not induced unless an *immunization* (resulting from blood transfusion or pregnancy) takes place. During the birth process, small amounts of the child's blood often enter the mother's bloodstream. Should the child's blood cells have paternal antigens, which are lacking in the mother's blood, his or her blood will effectively 'immunize' the mother. Should IgG antibodies develop they will represent a potential risk during *subsequent pregnancies* should the fetus once again present the same antigen. The resulting clinical picture is known as *morbus hemolyticus neonatorum* or *erythroblastosis fetalis* ("immune hydrops fetalis").

Once immunization has occurred, *thus endangering future pregnancies*, genetically at risk children can still be saved by means of cesarean section and exchange blood transfusions. Should the risk of rhesus immunization be recognized at the end of the first pregnancy, immunization of the mother can be prevented by means of a *passive infusion* of antibodies against the child's antigen, immediately following the birth. This specific immunosuppressive procedure is an empirical application of immunological knowledge, although the precise mechanism involved is not yet been completely understood.

Other blood group systems. There are other additional blood group systems against which antibodies may be produced, and which can present a risk during transfusions. Thus, the crossmatch test represents an important measure in the avoidance of transfusion problems. Immediately prior to a planned transfusion, serum from the prospective recipient is mixed with erythrocytes from the prospective donor, and serum from the prospective donor is mixed

with erythrocytes from the prospective recipient. To ensure no reaction following transfusion, there should be no agglutination present in either mixture. Some potentially dangerous serum antibodies may bind to the erythrocytes causing opsonization, but not necessarily inducing agglutination. To check for the presence of such antibodies, anti-human immunoglobulin serum is added and should it crosslink such antibodies agglutination will result.

Type III: Diseases Caused by Immune Complexes

Pathologies initiated by immune complexes result from the deposition of **small, soluble, antigen-antibody complexes** within tissues. The main hallmark of such reactions is *inflammation* with the involvement of complement. Normally, large antigen-antibody complexes (that is, those produced in equivalence) are readily removed by the phagocytes of the reticuloendothelial system. Occasionally, however—especially in the presence of *persistent bacterial, viral, or environmental, antigens* (e.g., fungal spores, vegetable or animal materials), or during *autoimmune diseases* directed against autoantigens (e.g., DNA, hormones, collagen, IgG) where autoantibodies to the body's own antigens are produced continuously—deposition of antigen-antibody complexes may become widespread often being present on active secretory membranes and within smaller vessels. Such processes are mainly observed within infected organs, but can also occur within kidneys, joints, arteries, skin and lung, or within the brain's plexus choroideus. The resulting inflammation causes local tissue damage. Most importantly, activation of complement by such complexes results in production of inflammatory C components (C3a and C5a). Some of these *anaphylatoxins* cause the release of vasoactive amines which increase vascular permeability (see also p. 103f.). Additional chemotactic activities attracts *granulocytes* which attempt to phagocytize the complexes. When these phagocytes die, their lysosomal hydrolytic enzymes are released and cause further tissue damage. This process can result in long-term chronic inflammatory reactions.

There are two basic patterns of immune complex pathogenesis:

- **Immune complexes in the presence of antigen excess.** The acute form of this disease results in *serum sickness*, the chronic form leads to the development of arthritis or glomerulonephritis. Serum sickness often resulted from serum therapy used during the pre-antibiotic era, but now only occurs rarely. Inoculation with equine antibodies directed against human pathogens, or bacterial toxins, often induced the production of host (human) antibodies against the equine serum. Because relatively large amounts of equine serum were administered for such therapeutic purposes, such therapy would result

in the induction of antigen-antibody complexes—some of which were formed in the presence of antigen excess—and occasionally induced a state of shock.

Immune complexes in the presence of antibody excess. The so-called *Arthus reaction* is observed when an individual is exposed to repeated small doses of an antigen over a long period of time, resulting in the induction of complexes and an antibody excess. Further exposure to the antigen, particularly dermal exposure, induces a typical reaction of edema and erythema which peaks after three to eight hours and disappears within 48 hours, but which sometimes leads to necrosis. Arthus-type reactions often represent occupational diseases in people exposed to repeated doses of environmental antigens: farmer's lung (thermophilic *Actinomyces* in moldy hay), pigeon breeder's lung (protein in the dust of dried feces of birds), cheese worker's lung (spores of *Penicillium casei*), furrier's lung (proteins from pelt hairs), malt-worker's lung (spores of *Aspergillus clavatus* and *A. fumigatus*).

Type IV: Hypersensitivity or Delayed Type, Cell-Mediated Hypersensitivity

Intracutaneous injection of a soluble antigen derived from an infectious pathogen induces a delayed dermal thickening reaction in those people who have suffered a previous infection. This *delayed skin reaction* can serve as a test to confirm immunity against intracellular bacteria or parasites.

For most cases, the time between administration of the antigen and the swelling reaction is 48–72 hours—as described above for cellular delayed type hypersensitivity (DTH) reactions in the skin (p. 99). As observed for antibody-dependent hyper-reactions of types I-III, the type IV response is pathogenic and *differs from protective immune responses only in terms of the extent and consequences of the tissue damage, but not in terms of the mechanism of action*. The balance between autoimmune disease and type IV immunopathology in such cases is readily illustrated by type IV reactions (e.g., aggressive hepatitis in humans or lymphocytic choriomeningitis in mice). Should the causal infectious pathogen be known, the response is termed a type IV reaction, if the causal agent is unknown (or not yet determined) the same condition may be termed "autoimmune disease." The reader is referred to the many examples of type IV responses already discussed within various chapters (DTH [p. 99], immune protection and immunopathology [Tables 2.**9** and 2.**10**, pp. 104 and 105], transplantation immunology [see below], and autoimmunity [p. 110ff.]).

Autoimmune T cells are usually directed against autoantigens that would otherwise be ignored (since they are only expressed in the extralymphatic periphery). Autoaggressive $CD4^+$ T cells apparently respond against myelin

basic protein in *multiple sclerosis*, against collagen determinants in *polyarthritis*, and against islet cell components in *diabetes*.

Transplantation Immunity

■ Transplant rejection within the same species is largely a consequence of MHC-restricted T-cell recognition of foreign MHC antigens. Interspecies rejection is additionally contributed to by antibodies, and intolerance between complement activation mechanisms. Methods for reducing, or preventing, rejection include general immunosuppression, tolerance induction by means of cell chimerism, and sequestering of the transplanted cells or organ. ■

The *strong* transplantation antigens are *encoded within the MHC complex* (see p. 58ff.), whilst the *weak* antigens constitute the MHC-presented allelic differences of *non MHC-encoded host proteins or peptides*. It is possible to differentiate between the **host-versus-graft** (HVG) reaction of the recipient against a genetically foreign tissue or organ, and the **graft-versus-host** (GVH) reaction.

The GVH reaction. This type of reaction results when *immunologically responsive donor T cells* are transferred to an allogeneic recipient who is unable to reject them (e.g., following a bone marrow transplant into an immuno-incompetent or immuno-suppressed recipient). The targets against which the transplanted T cells generate an immune response include the MHC class I and II molecules of the recipient. The recipient's transplantation antigens also present allelic variants of recipient self-peptides, which can be recognized by donor T cells as weak transplantation antigens when presented by common MHC alleles (it is conceivable that strong recipient transplantation antigens could be accepted and processed by donor APCs, however even if this did occur it would be of limited functional consequence as they would not be presented by the recipient APC in the correct antigen configuration). Weak histocompatibility antigens—for instance those peptide variants recognized as nonself when presented in combination with essentially histocompatible MHC molecules—play a more significant role in bone marrow transplants. The existence, and pathological role, of weak transplantation antigens has only been demonstrated in completely histocompatible siblings or within inbred animal strains with identical MHC. The *wide variety of alloreactive T cells* can be explained by cross-reactivity, as well as by the enormous number of different combinations of MHC molecules and cellular peptides. It must be emphasized that allogeneic MHC antigens on APCs and lymphocytes (so-called passenger lymphocytes) derived from the donor organ are particularly immunogenic since they express high levels of antigens and can traffic to

secondary lymphatic organs. Indeed the same foreign transplantation antigens are hardly immunogenic when expressed on fibroblasts or on epithelial or neuroendocrine cells, unless these cells are able to reach local lymphoid tissue.

To avoid a GVH reaction in immunoincompetent or suppressed bone marrow recipients, immunocompetent T cells must first be eliminated from the transplanted bone marrow. This can be achieved by using anti-T-cell antibodies, anti-lymphocyte antisera, and complement or magnetic bead cell-separation techniques. However, it is noteworthy that complete elimination of mature T cells leads to a reduction in the acceptance rate for bone marrow transplants, and that it may also weaken the anti-tumor effect of the transplant (desirable in leukemia). It seems that the small number of T cells transplanted with the bone marrow can mediate a subclinical GVH reaction, thus preventing rejection of the transplant but retaining the ability to destroy the recipient's leukemia cells and preventing tumor re-emergence.

Bone Marrow Transplants Today

- Reconstitution of immune defects involving B and T cells
- Reconstitution of other lymphohematopoietic defects
- Gene therapy via insertion of genes into lymphohematopoietic stem cells
- Leukemia therapy with lethal elimination of tumor cells and reconstitution with histocompatible, purified stem cells, either autologous or allogenic.

HVG reactions, that is immune responses of the recipient against transplanted cells or organs, are not generated in autotransplants (for instance transplantation of skin from one part of the body to another on the same individual). This also applies to transplants between monozygotic twins or genetically identical animals (**syngeneic transplants**). However, transplants between non-related or non-inbred animals of the same species (**allogeneic transplants**), and transplants between individuals of different species (**xenogeneic transplants**) are immunologically rejected. Because T cells recognition is subject to MHC restriction, cellular rejection within a species is even more pronounced than between different species, although the latter procedure involves other transplantation complications. These include the occurrence of natural *cross-reactive antibodies,* and a *lack of complement inactivation* by anti-complement factors (which are often species-incompatible and therefore absent in xenogeneic transplants), which together often results in hyperacute rejection within minutes, hours, or a few days—that is before any specific immune responses can even be induced.

Three types of transplant rejection have been characterized:

- **Hyperacute rejection** of vascularized transplants, occurring within minutes to hours and resulting from preformed recipient antibodies reacting

against antigens present on the donor endothelium, resulting in coagulation, thromboses, and infarctions with extensive necrosis.

- **Acute rejection**, occurring within days or weeks. This is accompanied by a perivascular and prominent occurrence of T lymphocyte infiltrates. Acute rejection can be prevented by immunosuppression.

- **Chronic rejection,** occurring within months to years. This is caused by low-level chronic T-cell responses, and can be mediated by cellular and humoral mechanisms. This can include obliterative vascular intima proliferation, vasculitis, toxic, and immune complex glomerulonephritis.

Antigenicity and Immunogenicity of MHC in Organ Transplants

A thyroid gland from donor "a," freshly transplanted under the renal capsule of an MHC (H-2)-incompatible recipient mouse "b" is acutely rejected (within seven to nine days). If the organ is treated in such a way as to kill the migratory APCs and leukocytes before it is transplanted, then transplant "a" will be accepted by recipient "b" (often permanently). However, should fresh spleen cells (APCs) from donor "a" be transferred by infusion 100 days later into the recipient "b," the previously accepted transplant "a," can sometimes be acutely rejected (i.e., within 10 days).

This experiment demonstrates that it is not the MHC antigens per se that are potently immunogenic, but rather that they only show this immunogenicity when they are located on cells capable of migration to local lymph nodes. Methods of implanting foreign tissue cells or small organs strictly extralymphatically, without inducing immune responses, are currently undergoing clinical trials (i.e., with islet cells in diabetes and neuronal cells in parkinsons disease).

Methods of measurement. The main methods used for follow-up analysis of HVG and GVH reactions are biopsies and histological evaluation, evaluation of blood cells and in-vitro mixed lymphocyte reactions (p. 132).

Immune Defects and Immune Response Modulation

- **Immune defects** are frequently acquired by therapy or viral infections, or as a consequence of advanced age. In rare cases immune defects can also result from congenital defects, these include severe combined immunodeficiency's (SCID) or transient partial immune defects (mainly involving IgA responses). **Immunomodulation** can be attempted using interleukins or monoclonal antibodies directed against lymphocyte surface molecules or antigenic peptides. **Immunostimulation** is achieved using adjuvants or

the genetically engineered insertion of costimulatory molecules into tumor cells. **Immunosuppression** can be induced globally using drugs, or specifically using antibodies, interleukins or soluble interleukin receptors; this can also be achieved by means of tolerance induction with proteins, peptides, or cell chimerism.

Immune Defects

The most important and frequent immune defects are *acquired*, e.g., iatrogenic (cytostatics, cortisone, irradiation, etc.), age-induced, or the result of viral infections (above all HIV). *Congenital defects* are rare; examples include Bruton's X-chromosome-linked B-cell defect, thymic hypoplasia (DiGeorge), and combined T- and B-cell deficiency resulting from MHC defects (bare lymphocyte syndrome) or from enzyme defects (adenosine deaminase [ADA] deficiency or purine nucleoside phosphorylase [PNP] deficiency). These defects can also be repaired by reconstitution (thymic transplants), or in some cases through the use of stem cells (gene therapy; one of the very first successful gene therapies was the treatment of ADA deficiency). More frequent congenital defects involve selective deficiencies, for example a relative-to-absolute IgA deficiency, normally being more prominent in infants than later in life. Children with such deficiencies are more susceptible to infection with *Haemophilus influenzae*, pneumococci, and meningococci. *General consequences of immune defects* include recurring and unusual infections, eczemas, and diarrhea.

Immunoregulation

This area of immunology is difficult to define and remains elusive. Antigens represent the most important positive regulator of immunity; since there is simply no immune stimulation when antigens have been eliminated or are absent. Other important regulators include interferon gamma (IFNγ) for TH1 responses, and IL-4 for TH2 responses. Further IL-dependent regulatory functions are in the process of being defined. The existence of specific $CD8^+$ T suppressor cells, capable of downregulating immune responses, has been postulated and their role was assumed to be that of counteracting the inflammatory $CD4^+$ T cell response. However, to date there has been no convincing proof of their existence. The term $CD8^+$ T suppressor cells, which is used frequently, is therefore misleading and inaccurate. In relatively rare cases, cyto-

toxic $CD8^+$ T cells do exercise a regulatory effect by lysing infected APCs or B cells (see also p. 106). It is unclear whether $CD4^+$ T cells could have similar effects. Regulation via *idiotypic/anti-idiotypic antibody networks* (i.e., antibodies directed against the ABS of other antibodies), or anti-TCR networks, have also been postulated—but remain hypothetical. Although attractive hypothesis, for most cases such regulatory pathways have only proved disappointing theoretical concepts, and as such should no longer be employed in the explanation of immunoregulation. In isolated cases, anti-idiotypic, or anti-TCR peptide-specific feedback, mechanisms can be modeled under forced experimental conditions. However such conditions probably fail to model normal situations, therefore they cannot accurately indicate whether these feedback mechanisms have a role in regulating the immune system as a whole.

Immunostimulation

The aim of immunological treatment of infections and tumors is to enhance immune responsiveness via the use of thymic hormones (thymopoietin, pentapeptides), leukocyte extracts, or interferons. Derivatives or synthetic analogs of microorganisms such as BCG, components of *Corynebacterium parvum* and peptidoglycans (e.g., muramyl peptide), or oligonucleic acids (CpG), are used as *adjuvants*. Components of streptococci and *Streptomyces*, eluates and fractions of bacterial mixtures, and the related synthetic substance levamisole are also used. The role of Toll-like receptors in these adjuvant effects is becoming increasingly understood, with a major role of these molecules being to link non-specific innate resistance to specific immunity. .

Recently developed immune therapy strategies aim to improve antigen presentation. For instance *interleukins,* or *costimulatory molecules* such as B7 or CD40, have been inserted into tumor cells by means of transfection. Hybrid antibodies have been constructed in an attempt to improve antigen recognition and phagocytosis (one such example is the coupling of an anti-CD3 antibody with tumor antigen-specific antibodies). Other ideas tested successfully in model experiments include systemic treatment with interleukins (this presents with frequent toxicity problems) or targeted insertion of GM-CSF, TNF, or IL-2. Alternatively, the production of IFNγ or IFNβ by cells, or the use of molecules capable of polyclonal T- and B-cell stimulation has been employed. This concept utilizes local chronic or acute infections with the aim of achieving inflammation surrounding, or direct infection of, tumor cells resulting in their cytolytic destruction. Such concepts have also been used to force phagocytosis and uptake of antigens by APCs with the aim of inducing or enhancing tumor immunity (e.g., BCG infections in bladder carcinoma treatment).