Identifying Strategies for Immune Intervention

Antonio Lanzavecchia

In recent years the molecular basis of antigen recognition by T cells has been unraveled and the various pathways that control T cell activation and functional specialization have been defined. Consequently, it is now possible to delineate various strategies for intervention with the immune system to design protective vaccines, to induce an effective response to tumor antigens, and to control graft rejection and autoimmune diseases.

For many decades, before any information on the immune system was available, physicians had recognized the phenomenon of specific immunity and exploited it to provide protection from infectious and toxic agents. In recent years, the elucidation of cellular and molecular components and the use of new experimental approaches have started to reveal the underlying working principles of the immune system. Consequently, it is now possible to identify new areas and new strategies for intervention. In this review I will examine the different control points of the T cell immune response, which correspond to possible levels of intervention, and discuss new and old approaches to immunotherapy.

Antigen Recognition by T Cells

T lymphocytes play a central role in the immune response by killing infected cells, controlling inflammatory responses, and helping B lymphocytes to make antibodies. Unlike antibodies that recognize antigens as such, the T cell receptor (TCR) recognizes antigen as a complex of a short peptide bound to a major histocompatibility complex (MHC) molecule on the surface of another cell called antigen presenting cell (APC). There are two types of MHC molecules: class I, expressed on all cells, and class II, which is expressed on macrophages, dendritic cells, B cells, and occasionally on other cell types. Much has been learned about the MHC-peptide complex from x-ray crystallography (*1*), peptide binding studies (*2*), and the analysis of MHC biosynthesis and antigen processing in living cells (*3*).

The MHC molecules are highly polymorphic. Different allelic forms have distinct peptide binding specificities. The sequencing of peptides eluted from MHC molecules has revealed allele-specific motifs, which correspond to critical anchor residues that fit into specific pockets of MHC molecules (*4*).

Newly synthesized MHC molecules are exposed to peptides in distinct intracellular compartments (*3*). Peptides derived from the degradation of cytosolic proteins are transported into the endoplasmic reticulum where they bind to nascent class I molecules. Peptides generated along the endocytic pathway bind to newly synthesized class II molecules which are specifically targeted to this compartment.

Although there are exceptions (*5*), class I–class II discrimination based on selective sampling of peptides makes biological sense. In this way $CD8^+$ cytotoxic T lymphocytes (CTLs), which recognize class I molecules, will kill virus infected cells that synthesize viral proteins (*6*), whereas $CD4^+$ T helper (T_H) cells, which recognize class II molecules, will stimulate selectively those cells that have captured antigen, for instance, antigen-specific B cells (*7*).

Although intact proteins need to be processed to generate antigenic peptides, soluble peptides can bind directly to a small fraction of empty class I or class II molecules present on the cell surface (*8*). These are important potential targets for immune intervention because they can be used to present exogenously added peptides to T cells.

T Cell Activation, Inactivation, and Exhaustion

T cell activation is controlled at several levels. The antigen specific trigger is the TCR, which is assisted by the CD4 or CD8 coreceptors (*9*). Ligation of the TCR by specific peptide-MHC complexes (signal 1) is sufficient to trigger effector function in already activated T cells, but in order to proliferate and acquire full effector function, naïve T cells and some activated T cells need to receive additional signals (signal 2 or costimulation) (Fig. 1). Signal 1 in the absence of signal 2 results in T cell inactivation or "anergy", which is associated with a block of interleukin-2 (IL-2) gene transcription (*10*). Anergy induction is blocked by cyclosporin A and can be counteracted by IL-2 or costimulatory signals. Signal 2 is antigen nonspecific and is delivered by CD28 or CTLA4 on T cells that interact with a ligand, B7, present on specialized APCs that are therefore called "professional" APCs (*11*). Ligation of CD28 induces increased transcription and stabilization of lymphokine mRNA in responding T cells (*10*).

The B7 molecule is expressed on macrophages and on dendritic cells (DCs), the prototypic professional APCs, and also on activated B and T lymphocytes. The DCs (*12*) function as "sentinels", because they reside in nonlymphoid organs where they pick up and process antigen and subsequently move to the T cell areas of lymph nodes and spleen, where they express high costimulatory capacity. The DCs thus provide optimal conditions for antigen delivery and for activation of naïve T cells recirculating in these areas.

The ability to follow the fate of naïve antigen-specific T cells in vivo with the use of TCR transgenic mice or superantigens has

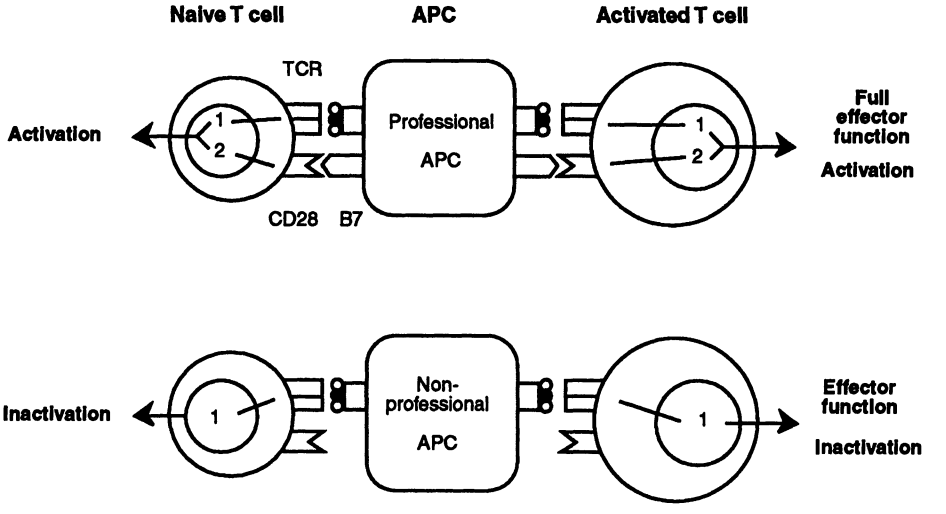

Fig. 1. The outcome of specific antigen recognition by T cells (signal 1) is determined by costimulation delivered by professional APCs (signal 2).

The author is a member of the Basel Institute for Immunology, CH-4005 Basel, Switzerland.

demonstrated that the outcome of antigen encounter depends on the type of APC and the intensity of the stimulation. Naïve T cells that encounter antigen on nonprofessional APCs become tolerant by anergy (*13*) or by TCR (*14*) or coreceptor down-regulation (*15*). Another possible outcome of T cell stimulation in vivo is T cell exhaustion, where T cells are transiently activated by very high concentrations of virus or superantigen and then rapidly die (*16*). These findings give a plausible explanation to old phenomena. The induction of anergy by nonprofessional APCs explains the failure to reject grafts depleted of DCs (*17*) as well as the phenomenon of low and high zone tolerance (*18*), in which presentation of soluble antigen may be done by resting B cells that are B7 negative (*19*). T cell exhaustion may explain the veto phenomenon (*20*) and the virus carrier state (*21*).

A complete molecular explanation for anergy, downregulation, and exhaustion is not yet available. One way to interpret these findings is to consider anergy and programmed cell death (apoptosis) as the default pathway of T cell activation and proliferation and differentiation as the rescue pathway, that is dependent on delivery of specific survival signals from other cells or soluble factors (*22*). CD28 and CD40 are examples of two signaling pathways that regulate clonal expansion in T and B cells, respectively. Several other surface molecules may play an important role, for instance, the *fas* receptor that can induce apoptosis and may play a role in the control of T cell clonal size and memory (*23*). These receptors and their signaling pathways may become in the future targets for therapeutic intervention in lymphoproliferative and autoimmune diseases.

Because we are beginning to understand the mechanisms of T cell activation, it is possible to work out some general principles. To optimize the induction of a T cell response one may use low amounts of antigen targeted onto professional APCs. Anergy induction can be counteracted using IL-2 or costimulation. To inhibit a T cell response one can consider two possibilities: (i) to block signal 1 at the level of the MHC-peptide–TCR-coreceptor interaction or at the level of signal transduction or (ii) to give signal 1 in the absence of signal 2, for instance by presenting antigen on nonprofessional APC or by blocking signal 2. These possibilities will be considered later in greater detail.

Functional Specialization

The three main effector functions of T cells, namely the capacity to help B cells, to induce inflammatory reactions, and to kill, depend on the type of lymphokines produced and on the presence of helper or lytic machinery. Effector T cells must also migrate to relevant sites and this capacity is regulated by the expression of adhesion molecules. Effector function and migratory capacity are acquired only after T cell activation and can be substantially influenced in their quality by signals from the environment.

When triggered by antigen, naïve T cells produce IL-2 only, which acts as an autocrine growth factor, but after clonal expansion they become competent to produce a variety of lymphokines. Under the influence of surrounding lymphokines, activated T cells may differentiate further to T_H1 or T_H2 cells (*24*). T_H1 cells produce IL-2 and interferon-γ (IFN-γ), induce inflammatory responses, and help production of opsonizing antibodies that bind to Fc-receptors on phagocytes. T_H2 cells produce IL-4, IL-5, and IL-10 and help production of nonopsonizing immunoglobulin G (IgG), IgA, and IgE antibodies. Different patterns of lymphokine production are also observed in $CD8^+$ T cells, which may thus exert distinct regulatory functions (*25*). In addition, some T_H1 cells may acquire cytotoxic activity and thus kill antigen-specific B cells (*26*).

Many immune responses to infectious agents or allergens polarize toward either T_H1 or T_H2, causing protection or immunopathology. This polarization occurs because T_H1 and T_H2 cells make lymphokines that suppress the development and the effector function of cells of the reciprocal type (*27*). For example, in resistant mouse strains, viable Leishmania parasites stimulate T_H1 cells that clear the pathogen by activating nitric oxide production in infected macrophages. In contrast, in susceptible strains, T_H2 cells that recognize dead parasites induce a more severe disease by suppressing macrophage activation through IL-4 (*28*). Remarkably, in vivo neutralization of IL-4 with a specific antibody blocks the T_H2 response and cures the disease (*29*). In several infections, such as leprosy, tuberculosis, and AIDS (acquired immunodeficiency syndrome), the protective T_H1 response may be similarly inhibited by the development of T_H2 cells (*30*). In other conditions however, T_H2 responses and IgE play a protective role, whereas inflammatory responses may cause immunopathology (*31*).

By producing different lymphokines that act as "switch factors", T cells can determine the isotype and therefore the effector function of the antibody. IL-4, transforming growth factor–β (TGF-β), and IFN-γ stimulate production of IgE, IgA, and opsonizing antibodies, respectively (*32*).

What decides between T_H1 and T_H2 responses? The type of APC class II molecules and antigen dose (*33*) can be important discriminatory factors, but a major role is played by lymphokines, especially IL-4, which is required for the development of a T_H2 response (*34*). IL-10 (*35*) and TGF-β (*36*) also favor T_H2 responses through their effects on APCs. TGF-β, which is present in amniotic, cerebrospinal, and ocular fluids and in the gut, is responsible for the "immune deviation" (from T_H1 to T_H2) characteristic of the T cell responses in these privileged sites (*36*). Oral administration of antigen induces $CD8^+$ antigen-specific T cells that produce TGF-β and suppress bystander inflammatory responses mediated by T_H1 cells (*37*). In addition, TGF-β can directly affect T cells by down-regulating integrins that favor homing to the brain or inflamed tissues (*38*) and up-regulating integrins responsible for homing to the gut (*39*). Cytokines produced by phagocytic cells stimulated in early stages of the immune response such as IFN-α and IL-12 can influence the T cell differentiation by steering the balance toward T_H1 (*40*).

The functional specialization of T cells provides an enormous opportunity for immune intervention. In principle, one may be able to exploit the reciprocal regulation of T_H1 and T_H2 to induce protective responses to infectious agents, to suppress IgE and enhance IgA or opsonizing antibody production, and to contrast autoimmunity. In practice, although some tools such as recombinant cytokines, soluble receptors, and blocking antibodies are available their therapeutic effect may not be always clearly predictable. This is because cytokines function only locally and their effect is dependent on the presence of other cytokines and soluble inhibitory receptors in the local microenvironment.

With knowledge of how T cells recognize antigen and how they respond to stimulation in various circumstances, we can begin to envision ways by which the T cell response can be manipulated. Targets for immune intervention exist at many levels, as is discussed below.

Vaccination

The goal of vaccination is the induction of protective immunity. The target was once limited to infectious diseases, but has now broadened to include treatment of tumors, allergy, and even autoimmune diseases. A rational approach to vaccination must involve three steps: (i) the identification of the protective effector mechanisms, (ii) the choice of an antigen that can induce a response in all individuals, and (iii) the use of an appropriate way to deliver the vaccine so that it will induce the right type of response.

In some cases vaccination may result in exacerbation of the disease. The distinction between protective, useless, and dangerous responses is essential for vaccine design and can be facilitated by the availability of new

methods of analysis and new strains of mice in which individual components of the immune system, such as MHC molecules or cytokines, have been knocked out by homologous recombination.

The second problem, that is, the choice of the antigen, relates to the variability of the host and of the pathogen. The capacity to respond to antigen can be influenced by general health, but is mainly determined by genetic MHC polymorphism. The extensive polymorphism of MHC in an outbred population contributes to the recognition by different individuals of different epitopes in a complex protein antigen. This advantage for the species constitutes a problem for the design of vaccines based on short protein sequences. To effectively vaccinate a population, a vaccine must therefore contain epitopes that can be processed and bind to at least one allele in every individual.

The identification of T cell epitopes is critically dependent on the availability of antigen-specific T cell clones. Epitopes can be mapped by testing the capacity of T cell clones to respond to overlapping synthetic peptides spanning the whole protein. The identification of sequences that conform to known class I or class II binding motifs may facilitate this search (*41*), but cannot substitute for T cell clones because flanking regions can influence processing and binding to class I and class II MHC molecules in as yet unpredictable ways (*42*).

Some class I alleles are present at high frequencies and thus it may be possible to make a class I vaccine effective in up to 90% of the population using only a few epitopes. For class II molecules, some short peptides are universally immunogenic either because they contain "nested" epitopes that bind to several alleles (*43*) or because they contain one epitope that interacts with conserved subsites of class II molecules (*44*). These epitopes are particularly useful if they coincide with regions of limited variability of the pathogen (*45*).

The inherent risks of vaccines containing few T and B cell epitopes are that they may fail to induce a response in all individuals or they may allow the selection of escape mutants. Nonresponse and escape do not appear yet to be a major problem for the small hepatitis B vaccine (*46*). Escape, however, is a major problem for highly variable pathogens that give sustained infections such as the human immunodeficiency virus (HIV) (*47*) or hepatitis C virus (*48*), where escape variants are continuously selected in infected donors. It is therefore rational to try to generate immunity to T and B cell epitopes that show less or no variability.

Once the protective mechanism and the antigen are known, the appropriate immunization method must be identified to induce either antibody, T_H1, T_H2, or CTL responses. The first step in the induction of an antibody response is the activation of T helper cells by presentation of antigen on DCs, a step facilitated by the use of an appropriate adjuvant. Effector T helper cells can subsequently interact with the specific B cells that have captured native antigen on their membrane Ig and then present antigenic peptides on their MHC molecules. In T-B collaboration, the B and T cell epitopes must be physically linked to be cointernalized by the B cell, but do not necessarily need to be part of the same molecule (*7, 49*). This leaves a large (but not unlimited) degree of flexibility to arrange B and T epitope, for instance, by coupling a "carrier" protein to bacterial polysaccharides one can induce a T-dependent antibody response to carbohydrates.

Besides their protective effect due to antigen neutralization and opsonization, antibodies can be used to augment or suppress T cell as well as antibody responses. Soluble IgG complexed with antigen can increase antigen capture and presentation by professional APC that carry Fcγ-receptors (*50*). The same complexes can localize antigen to the surface of follicular DCs in the germinal centers thus facilitating selection of specific B cells (*51*). Such soluble antibodies, however, can block antigen capture by specific B cells in an epitope-specific fashion and thus inhibit antibody response to the same epitope to which they bind (*7, 52*). Furthermore, antibodies can affect processing of the antigen to which they bind and suppress the generation of particular T cell epitopes in both B cells and professional APCs (*53*).

Antibodies can also be used to target antigen to cell surface molecules that deliver antigen to the class II processing pathway (*54*). The targeting of antigen to mouse DC surface molecules can prime T helper cells in the absence of adjuvant by maximizing the efficiency of T cell activation and minimizing the chance of anergy induction (*55*). Identification of DC-specific molecules that are suitable for targeting is necessary in order to pursue this strategy in humans. Antigen targeting to B cells using antigen–anti Ig conjugates has only a modest effect, probably because most B cells are resting and therefore unable to costimulate (*56*). This method, however, may be useful to suppress or modify the T cell response as discussed below in the case of autoimmune diseases.

An advantage in the use of short peptides as opposed to intact antigen is that peptides can selectively stimulate specific T cells without necessarily inducing an antibody response to the intact antigen. Because soluble peptides bind to surface MHC molecules, they will be presented by the more abundant nonprofessional APCs and may thus favor induction of anergy, exhaustion, or a T_H2 response. If appropriate conditions of immunization can be found, peptides may be used to modify or suppress unwanted antibody responses by their effect on specific T helper cells (*56a*). The induction of anergy in T helper cells may deprive B cells of help. Changing the lymphokine profile may change the isotype of antibody produced. Finally, induction of the lytic machinery may result in suppression of the antibody response by the killing of specific B cells (*26*).

Live attenuated viruses and infective or defective viral vectors (*57*) can be used as vaccines to deliver newly synthesized antigen to the class I processing pathway and induce a CTL response. However, these agents may have serious side effects. The injection of naked plasmid DNA results in the expression of the encoded antigen by muscle cells, and perhaps APCs, resulting in the induction of protective CTLs as well as antibody responses (*58*). If this method of "genetic immunization" proves to be safe, it may become a major breakthrough in vaccination because it can be used repeatedly to immunize to different antigens while avoiding the risk of an infectious virus and the problem of the immune response to the vector.

An alternative approach for CTL induction is either to use peptides to fill cell surface class I molecules or to deliver intact proteins to class I by alternative pathways. Peptides injected with adjuvant (*59*) or conjugated to a lipid (*60*) have been used to induce protective anti-viral responses. Furthermore, the simultaneous use of a class II–restricted peptide could increase the cytotoxic response (*61*). Possibly all of these features can be incorporated in a peptide vaccine that contains epitopes that bind to the most frequent class I alleles.

There are two additional mechanisms of antigen delivery to class I molecules that can be exploited to make vaccines for CTL. The first is used by bacteria such as *Listeria monocytogenes* that produce lytic toxins that allow their escape from phagosomes into the cytosol where they are processed by the conventional class I pathway (*62*). An alternative pathway involves the phagocytic processing of particulate antigens and the direct loading of peptides on mature empty class I molecules (*63*). This pathway that is characteristic of macrophages may be involved in class I–restricted responses to bacteria that lack escape mechanisms (*64*) as well as in the response to cell-associated antigens or minor histocompatibility antigens (*65*). It may be possible to exploit either one or both mechanisms to generate vaccines for CTL using bacterial vectors with or without the escape mechanism, an-

tigen in ISCOMS (immunostimulating complexes) (66) or in liposomes (67), noninfectious virus-like particles (68), and in general particulate or cell-associated antigens.

The development of adjuvants is an essential aspect of vaccination that has received relatively little attention. The mechanism of action of adjuvants, which have been developed through a totally empirical approach based on the capacity to stimulate antibody responses, may be related to their capacity to induce a slow and sustained release of antigen and to stimulate APCs by inducing the production of inflammatory cytokines (69). One can foresee the possibility of developing different adjuvants suited for the delivery of peptides and protein antigens to class I and class II molecules on professional APCs and at the same time able to stimulate APCs and other cells to produce cytokines that channel the response in the right direction. Cytokines such as tumor necrosis factor–α (TNF-α) and granulocyte-macrophage colony-stimulating facor (GM-CSF) may become an important part of future adjuvant technology.

Tumors

All the examples of vaccination discussed above involve the induction of an immune response to nonself antigens. A special case of vaccination is that against autologous tumor cells. There is now a large consensus that some tumor cells are antigenic in the sense that they express tumor-associated antigens (TAAs) that can be recognized by T cells in a syngeneic host (70), but are not immunogenic, because they lack costimulatory molecules and may produce suppressive cytokines.

There are at least two approaches to tumor vaccination. The first is to identify a TAA to be used as a vaccine, the second is to increase the immunogenicity of tumor cells and let the immune system decide which antigen to attack.

A method for the identification and cloning of genes encoding TAA recognized by CTL has been developed (71). Some TAA are encoded by normal nonmutated cellular genes that are highly expressed in tumor cells but not, or to a very minor extent, in normal cells. For example, MAGE-1 encodes an MHC class I HLA-A1–restricted epitope that is recognized by a melanoma-specific CTL isolated from an HLA-A1$^+$ patient (72). MAGE-1, which is expressed in high amounts in 40% of melanomas as well as other tumors and in low amounts in melanocytes and other tissues, might be used in an appropriate form (peptide, transfected APC, retroviral vector, or naked DNA) to develop a vaccine that may be effective in more than 10% of melanoma patients, that is, those that carry

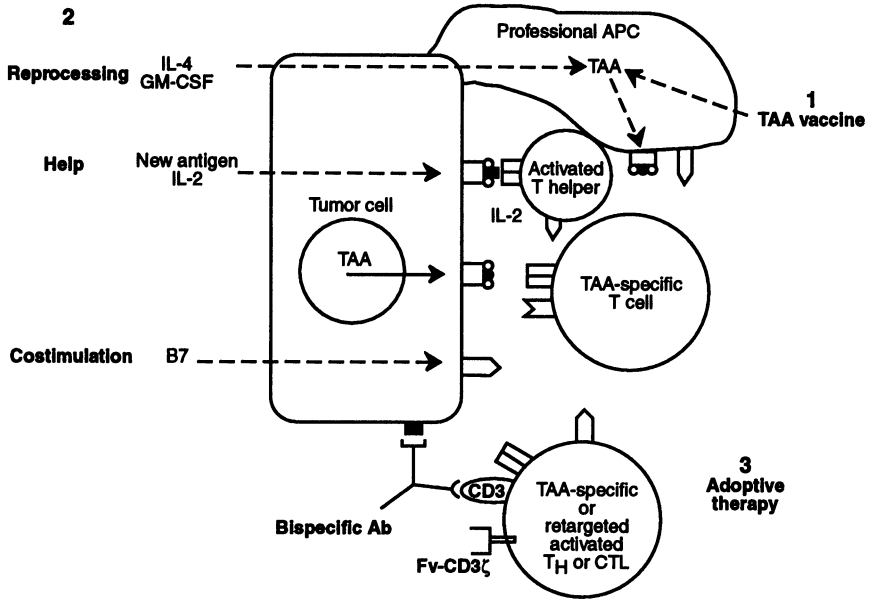

Fig. 2. Possible ways to boost the T cell response to a tumor associated antigen (TAA).

HLA-A1 (72). Other candidates for vaccine development are viral proteins (73), mutated oncogenes (70), and mutated or junctional Ig variable regions in B cell neoplasias (74).

The second approach to tumor vaccination does not require knowledge about either T cell epitopes or MHC haplotype and relies only upon attempts to facilitate recognition of tumor cells. This can be done in three ways: (i) by providing local T cell help, (ii) by representation of TAA on professional APC, or (iii) by delivering the missing costimulatory signal (Fig. 2). Many interventions take advantage of one or more of these features.

T helper cells can enhance CTL responses, especially when the stimulatory conditions are suboptimal (75). This provides a rationale to target T helper cells against tumor cells in the hope these will provide help (IL-2) or costimulation (B7) for the development of a cytotoxic response. This can be achieved by directly displaying new antigens on tumor cells by transfection, chemical modification, or by antigen targeting (54, 76).

Killed autologous tumor cells may provide a source of antigen for representation, but this mechanism requires strong activation of macrophages and DCs. Help or APC activation can be induced by cytokines, but the systemic administration of cytokines has serious side effects. Local injection of small doses of IL-2 or IL-4 can induce tumor rejection and specific memory (77). These results provided the rationale for using tumor cells transfected with lymphokine genes (78). In all cases injection of modified tumor cells induces a local inflammatory response that, in some cases, is followed by a systemic response leading to rejection of the wild-type tumor and the establishment of T cell memory. Transfection with the IL-2 gene may locally prevent anergy induction by tumor cells and thus expand TAA-specific T cells (79). Transfection with IL-4 gene leads to a strong inflammatory reaction and tumor infiltration with macrophages and eosinophils, followed by a TAA-specific T cell response (80). The protective mechanism may involve processing and representation of tumor antigen by professional APC, which are recruited by IL-4. This mechanism has been formally demonstrated for tumor cells transfected with GM-CSF, which recruits macrophages and DCs and induces a strong cytotoxic response against TAAs (81).

One application of our new molecular understanding of costimulation has been the transfection of tumor cells with the B7 gene (82). An active cytotoxic response against a mouse melanoma could be induced. In one study a CTL response against endogenous tumor antigens could be generated in the absence of T helper cells demonstrating that, when enough costimulation is available, no help is needed.

All these examples illustrate different mechanisms that may be used to enhance the anti-tumor response. Although many regimens induce protection in experimentally transplanted tumors it is not yet possible to extrapolate from these results what may happen in the case of spontaneous human tumors. In the latter case the anatomical location, the tumor mass, the compromised immune system and the long tumor-host relationship may contribute to making some of these approaches less effective. More physiological animal models and

better characterization of the human immune response to tumors and to tumor vaccines will help in finding the best vaccination approach.

Adoptive Therapy

So far we have considered various ways of actively stimulating the immune system. An alternative approach is to passively provide activated T cells or antibodies to the patient. Adoptive immunotherapy with antibodies or with antibodies coupled to toxins or drugs has been used (*83*).

A virus can escape by mutating the viral epitope that is recognized by antibody, while preserving the site required for binding to cellular receptors. These conserved sites can be targeted by using a recombinant soluble form of the cellular receptor as a simple decoy, or by linking it to Ig constant regions to provide antibody effector functions (*84*).

In the case of viral diseases or tumors, adoptive transfer of activated T cells may be considered, especially in immunosuppressed patients. Protective T cells, obtained from blood, tissues, or even from primary cultures (*85*), could be expanded in vitro and given back to the patient. In mouse models, adoptive transfer of virus-specific T cells can lead to virus clearance (*86*) and a single CTL clone specific for a viral tumor antigen can eradicate large tumors when given together with IL-2 (*87*). In humans, adoptive transfer of virus-specific CTLs results in reconstitution of the specific response in immunosuppressed patients (*88*). Attempts to attack established tumors by transfer of in vitro expanded tumor infiltrating lymphocytes or lymphokine-activated killer cells have led to variable results and considerable side effects probably due to low specificity and simultaneous use of high doses of IL-2 (*89*). Improved methods for the in vitro selection of specific T cells based on the precise knowledge of TAAs and the identification of culture conditions that maintain the desired effector function and migration capacity are required before the full potential of adoptive immunotherapy can be assessed.

An additional possibility is to retarget T cells by coating them with bispecific antibodies that bind CD3 and any antigen of choice, for instance a surface molecule highly expressed on tumor cells. Bispecific antibodies or recombinant "janusins" can target T cells to ovarian carcinomas or to HIV-infected cells (*90*). It is also possible to transfect T cells with genes that encode artificial receptors, for instance chimeric molecules consisting of an extracellular antigen-binding domain [CD4, CD8, or the antibody variable fragment (Fv)] linked to the intracellular portion of CD3ζ chain which transduces the triggering signal (*91*).

In principle, cultured T cells could also be transfected with genes that confer new effector functions (for instance a lymphokine) or replace a missing function (for instance the CD40 ligand to correct its genetic defect). However, this may be a quite difficult task, because expression of these genes must be correctly regulated after T cell activation and this may require transfection of very large DNA fragments.

Blocking Versus Suppression

In the context of immune intervention, the target of suppression would be pathogenetic T cells, usually T_H1 cells or CTLs that are responsible for graft rejection or autoimmune disease. The course of the disease can be altered by blocking T cells or changing their properties. Whereas selective blocking is difficult to achieve and is limited in its effect, the diversion of the immune response, as we will discuss, may achieve long-lasting and more widespread effects.

Transduction of signal 1 can be blocked by cyclosporin A, an immunosuppressive drug that is used to prevent graft rejections. Although extremely efficient, this treatment lacks selectivity and causes generalized immunosuppression. Antibodies to the CD3-TCR complex can inhibit T cell responses, but lack selectivity and have considerable side effects. A more selective inhibition might be achieved by antibodies to the variable (V) regions of the T cell receptor, such as V_β (*92*) or by "idiotypic vaccination" (*93*). In the latter case, inactivated autoreactive clones or synthetic peptides corresponding to their TCR V region are used as vaccines. The mechanism responsible for the positive effects observed in experimental animal models (*94*) has not been clarified, although it is assumed to be mediated by T cells that recognize TCR peptides or even epitopes present only on activated T cells (*93*). The necessary prerequisite for the success of all approaches based on TCR structure is that the clonal population of disease-inducing T cells must display a TCR of limited diversity as it is the case in some animal models of autoimmunity. However, in human autoimmune diseases no consistent picture of restricted V gene usage has emerged that might justify optimism for this approach (*95*), nor is there any clear evidence for a T cell response to naturally presented TCR peptides.

Blocking of the coreceptor can lead to a more selective inhibition. Simultaneous administration of antigen together with nonlytic antibodies to CD4 and CD8 can induce a state of antigen-specific tolerance to soluble antigens and allografts (*96*). Even if the autoantigen is not known, as in diabetic NOD mice, tolerance can be established by briefly administering antibodies to CD4, without affecting the response to new or recall antigens (*97*). Transplantation tolerance induced by skin grafts under the umbrella of anti-CD4 and -CD8 involves an active suppressive mechanism, because $CD4^+$ cells from tolerant mice can induce tolerance in transfer experiments (*98*). The mechanism responsible for this phenomenon of "infectious tolerance" is not clear, because the specific tolerizing cells have not been isolated, but may be related to the diversion of T cells toward production of suppressive lymphokines. It will be important to understand how antibodies to CD4 change signal transduction and to explore possible synergistic effects with other treatments.

It is possible to block costimulation using a soluble form of CTLA4, the high affinity ligand for B7. In vitro, antigen stimulation in the presence of soluble CTLA4 induces a state of antigen-specific anergy and inhibits the production of IL-2 and IFN-γ but not of IL-4 (*99*) and may thus push the response toward T_H2. In vivo, however, the effects are less striking. Soluble CTLA4 prevents primary but not secondary antibody responses (*100*). Furthermore it prevents the rejection of xenografts (*101*), but has only a modest effect on survival of heart allografts (*102*). T helper cell priming for antibody responses does not appear to be impaired in transgenic mice expressing a soluble form of CTLA4 (*103*), which suggests the involvement of redundant costimulatory mechanisms (*104*).

Adhesion molecules are important targets for intervention. Antibodies to integrins can prevent migration of autoreactive T cells to the target organ (*105*). A short course of anti–LFA-1 (antibody to leukocyte function associated antigen–1) plus anti–ICAM-1 (antibody to intercellular adhesion molecule–1) can induce long-term survival of heart allografts and specific tolerance (*106*).

A specific target for intervention is the peptide-MHC complex. The rationale for this comes from studies that show associations between susceptibility to autoimmune diseases and certain MHC class II alleles (*107*). One interpretation of these findings is that the class II alleles present disease-inducing peptides to T cells. Indeed, blocking these class II molecules with antibodies prevents disease in experimental animal models (*108*). However, this treatment is not selective and when prolonged will have serious side effects.

Peptides that bind with high affinity to MHC molecules can, by competition, prevent experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis that is induced by immuniza-

tion with a myelin basic protein (MBP) peptide (*109*). However, any effect of soluble competitor peptide on presentation of physiologically processed antigen or preexisting complexes is unlikely. Indeed, exogenous peptides cannot efficiently compete for binding of processed antigen to newly synthesized class II molecules, nor can they displace preformed complexes, since the binding of peptides to class II molecules in living cells is practically irreversible (*110*).

All the approaches outlined above involve some form of blocking. An alternative strategy is to stimulate autoreactive T cells with the antigen to change their effector function from T_H1 to T_H2. An exploitable difference between T_H1 and T_H2 is that T_H2 do not seem to require costimulation, whereas T_H1 cells do (*111*). Consequently, presentation of antigen without costimulation may lead not only to anergy in T_H1 cells but also to stimulation of T_H2 responses and thus to IL-4–mediated immune regulation. Indeed, targeting antigen to resting B cells has been shown to favor T_H2 responses (*111*). An example relevant to autoimmune diseases is the targeting of an MBP peptide to B cells using peptide–anti-IgD conjugates (*112*). This treatment protects rats from induction of EAE using the same peptide in adjuvant. Protection is not due to anergy but rather to the stimulation of a different type of effector T cells with disease suppressing activity.

The immune deviation characteristic of gut-associated responses has been exploited in several experimental autoimmune diseases. Oral administration of an autoantigen can induce a down-regulation of the specific systemic inflammatory response. The effector cells are antigen-specific, $CD8^+$ T cells that produce TGF-β after stimulation with antigen and suppress bystander responses in vitro (*113*).

The use of peptides has opened new and unexpected possibilities of intervention, since the TCR is sensitive to small changes in the structure of the complex recognized. Single amino acid substitutions in an antigenic peptide can generate an analog that binds equally well to the MHC molecules but has a different capacity to trigger T cells. Some analog-MHC complexes behave as pure antagonists. They do not induce any signal in T cells, but when displayed on the same APC that is presenting the antigen, can block signal 1 by a mechanism that has not yet been characterized (*114*). In vivo, peptide antagonists are expected to exert an effect only in the presence of the agonist, that is, only on cells that present the self antigen. Since antagonists act on particular TCRs it is not clear how they might affect a polyclonal response.

Other analogs behave as "partial agonists." They trigger T cells but induce a response which is different from that elicited by the wild-type ligand (*115*). The effect of partial agonists may be more widespread, because they will be recognized on all APCs and therefore may not only change the T cell effector function, but also distract T cells from the site of disease. Finally, it is possible that a disease suppressive effect might be achieved in vivo even with an antigenic peptide, if this is given in a way that favors presentation by nonprofessional APCs, for instance in a soluble form (*116*).

Thus, inhibition of specific T cell activation at the site of disease could be achieved by pure antagonists, whereas partial agonists and antigenic peptides may distract, anergize or exhaust the specific T cells or may reprogram these cells to a different (suppressive) effector function. Presentation of self antigen in an appropriate context may also prime autoreactive regulatory T cells with disease suppressing activity. It is possible that one or more of these mechanisms might have been responsible for the successful treatment of EAE using antigenic peptides or analogs (*117*).

The fact that several treatments can "deviate" the immune response suggests the possible existence of a common final pathway for the induction of these regulatory T cells (Fig. 3). If this is the case, it will be important to identify the possible synergistic treatments that may render this strategy much more effective.

An additional level of intervention that is only partially exploited is the pharmacological modulation of signal transduction. In the future it may become feasible to modulate the pathways of costimulation, apoptosis, and differentiation to use drugs to control the immune response.

Finally, there are two fundamental aspects of autoimmune diseases that need to be considered. The first is the difference between experimental and spontaneous autoimmune diseases. Although the former are the consequence of an acute immune response to a well-defined antigen, spontaneous autoimmunity develops over several years and there is therefore time for the response to spread to several epitopes. If antigen-based desensitization has to be used, it will be essential to identify the autoantigens and the epitopes recognized. This will be possible by isolating the pathogenetic T cell clones and by the use of new ultrasensitive peptide sequencing methods (*118*).

The second aspect is that by understanding the mechanisms of disease we may get new hints for intervention. For instance, it will be important to know how intrinsic abnormalities of T, B, and other cells may contribute to the disease (*119*). The effector cells that mediate tissue damage also need to be identified. For instance, most of the T cells that produce lymphokines in lymph nodes and lungs of mice infected with influenza virus are not antigen-specific (*120*). These results imply that the cells that produce the bulk of the lymphokines at inflammatory sites are T cells that are nonspecifically recruited by cytokines or cell contacts. Understanding of the molecular mechanism of recruitment may thus provide a new target for intervention.

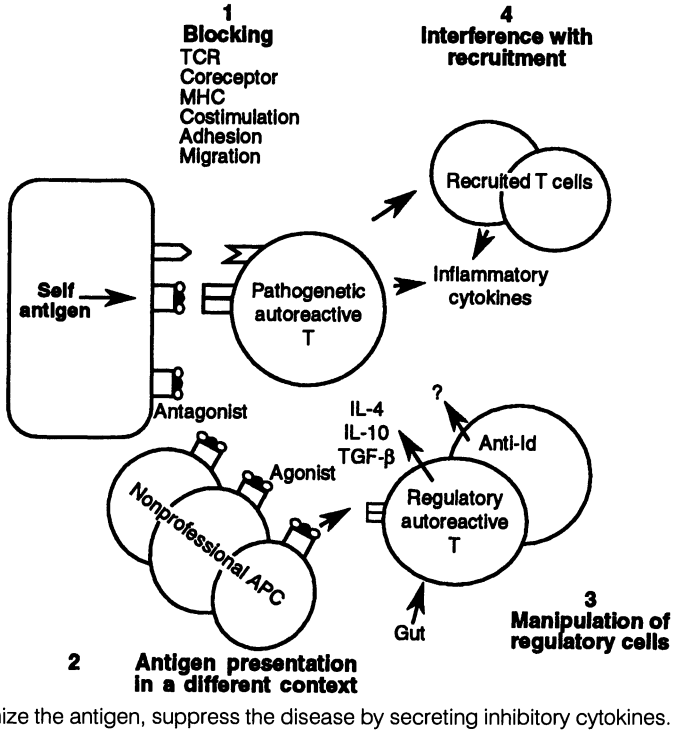

Fig. 3. Possible ways to control an autoreactive response. Autoreactive T cells triggered by a self antigen produce inflammatory cytokines and recruit other T cells. These cells can be blocked by various treatments with different degrees of selectivity (see text). Presentation of the self antigen or analog peptides on different APC may antagonize, distract, anergize, or exhaust autoreactive T cells and even change their effector function. At the same time, regulatory T cells may be primed by presentation of the self antigen in an appropriate context (gut or nonprofessional APC). These regulatory cells can migrate to inflammatory sites and, when they recognize the antigen, suppress the disease by secreting inhibitory cytokines.

Concluding Remarks

A biological phenomenon becomes more exploitable when we understand its molecular basis. In this sense the structure of the antigenic complex and its formation is fully understood and solved up to the level of the crystal structure of a peptide-MHC complex. Because we have acquired a precise methodology of antigen identification and we understand the general principles of antigen processing, we can now start to design better vaccines against pathogens, tumors, and perhaps allergic and autoimmune diseases.

The problem of the control of T and B cell function in vivo is more complex because it is integrated only at the level of the whole organism and therefore involves a number of variables that are still hard to estimate.

The costimulatory B7-CD28 pathway that was conceived to explain self-nonself discrimination (*121*) has been characterized at the cellular and molecular level and can now be exploited to induce an immune response to tumor cells. Similarly, we are starting to understand the molecular control of the development of T helper cell subsets, while mechanisms of exhaustion, recruitment, and suppression, which can be inferred on the basis of strong phenomenological evidence, still lack a molecular definition. From a better understanding of the molecular basis of immune regulation we may become able to fight cancer and autoimmunity using the immune system's own strategies.

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122. I thank S. Abrignani, G. de Libero, K. Karjalainen, P. Lane, and A. Livingstone for critical reading and discussion. The Basel Institute for Immunology was founded and is supported by F. Hoffmann–La Roche Ltd., Basel, Switzerland.