Harnessing the immune system to treat chronic infectious diseases or cancer is a major goal of immunotherapy. Among others, impediments to this aim include host failure to identify tumor antigens, tolerance to self and negative immunoregulatory mechanisms. But with recent progress, active and passive immunotherapy are proving themselves as effective therapeutic strategies.

Immunotherapy: past, present and future

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Active immunotherapy has been effective against agents that normally cause acute self-limiting infectious diseases followed by immunity. However, effective immunotherapy for chronic infectious diseases or cancer will require the use of appropriate target antigens; the optimization of the interaction between the antigenic peptide, the antigen-presenting cell (APC) and the T cell; and the simultaneous blockade of negative regulatory mechanisms that impede immunotherapeutic effects. Furthermore, passive immunotherapy using monoclonal antibodies and receptor Fc-fusion proteins has come of age and has shown great clinical success. Eleven monoclonal antibodies, including unmodified antibodies and antibodies armed with toxins or radionuclides, have been approved to prevent allograft rejection or to treat autoimmune diseases and cancer. An additional 400 monoclonal antibodies are in clinical trials.

Jenner's discovery that deliberate infection with cowpox virus caused mild disease and subsequent immunity to smallpox infection started one of the greatest revolutions in medical intervention (Fig. 1, Box 1). Preventive vaccines have been especially successful against infectious agents such as viruses, which cause self-limiting diseases that are normally followed by long-lasting immunity. However, it has taken recent insights into the nature of the relevant cells, cytokines and signaling pathways that both positively and negatively regulate immune responses to make progress in the immunoprevention and immunotherapy of established chronic infections with agents such as retroviruses, mycobacteria and parasites, as well as headway for cancer. A series of outstanding reviews have been published that focus on active immunotherapy[1–6]. Dramatic immunotherapeutic advances initially used passive approaches with antitoxin-containing antisera, but over the past 25 years have also used unmodified monoclonal antibodies and antibodies armed with toxins or radionuclides[7–11]. This review considers a few of the highlights of past approaches, with special reference to unusually exciting recent developments that suggest major strategies for the development of effective immunotherapies for the treatment of established infectious diseases and cancer.

Prophylactic and therapeutic vaccines

For over two centuries, active immunotherapeutic approaches have been at the forefront of efforts to prevent the infectious diseases that plague humankind. In eighteenth-century Europe, smallpox caused 10% of all deaths. In 1796, however, Edward Jenner used vaccination with cowpox to induce immunity to smallpox (Box 1). These endeavors culminated in the eradication of natural smallpox infection 180 years later. Using a parallel strategy involving killed or attenuated pathogens, effective vaccines were developed for acute self-limiting infectious agents such as rabies, typhoid, cholera, plague, measles, varicella, mumps, poliomyelitis, hepatitis B and the tetanus and diphtheria toxins. Active immunotherapy has been much less effective against cancer or chronic infectious diseases caused by agents that have developed strategies to escape normal immune responses.

Vaccines for chronic diseases

The idea that cancers can be treated by active immunization arose in the 1890s with the proposals of Paul Ehrlich and William Coley[12,13]. Although there has been only limited success with active immunization for established chronic infectious diseases or cancer in humans, recent immunological insights represent advances toward this challenge. One is the identification of tumor rejection antigens by defining tumor-associated antigens that stimulate T-cell responses (see Box 2)[1–6,14–18]. These include tumor-specific antigens, the results of mutations, viral antigens in cancers associated with viruses, and tumor-specific differentiation antigens. Viruses causally associated with neoplasias, including hepatitis B, hepatitis C (hepatoma), human papilloma viruses (cervical cancer), Epstein-Barr virus (Burkitt lymphoma, Hodgkin disease) and human T-cell lymphotrophic virus-1 (T-cell leukemia and lymphoma) have been shown to induce host immune responses. Each of these agents is being used in vaccines or is under study.

The immunoglobulin idiotype is an additional antigenic target that is present in B-cell lymphomas. After Eisen and colleagues[19] showed that the immunoglobulin idiotypes of myeloma proteins, which function as tumor-specific antigens, could be used in vaccines to select against myeloma cells that form intact myeloma proteins, Stevenson validated the efficacy of this target in mouse models[20]. In 1982, Levy and colleagues used anti-idiotypic monoclonal antibodies to induce remissions in patients with B-cell lymphoma, in the first effective use of monoclonal antibody therapy for a human malignancy[21,22]. Levy's group subsequently used the immunoglobulin idiotype as a target for vaccine therapy of B-cell lymphoma using both an idiotype granulocyte-macrophage colony-stimulating factor (GM-CSF) fusion protein and naked DNA immunization. More recently, effective anti-tumor responses were induced in 17 of 19 patients with B-cell lymphoma who received an idiotype-directed vaccine[23].

An alternative approach to defining cancer-associated antigens has been to identify antigens recognized by the tumor-bearing host. For example, the technique of serologic identification by recombinant expression cloning (SEREX) is used in cancer patients to identify circulating IgG that are specific to tumor antigens[14]. Screening cDNA libraries from tumor tissues using tumor-reactive T-cell lines and clones from cancer patients is another approach that could lead to cellular

tumor immunity rather than humoral immune responses[1–3,5,23]. An alternative approach is to characterize tumor-associated peptides bound to class I major histocompatibility (MHC) molecules by mass spectrometry[16]. Furthermore, the use of melanocyte-specific targets, including MAGE, MART-1/melanA, tyrosinase, tyrosine-related protein-1, and gp100, was associated both with antitumoral clinical responses and with vitiligo resulting from immune attack on both malignant and normal melanocytes[1,3,5,24]. The present intense efforts in molecular profiling of cancer should provide additional useful antigenic targets for immunotherapy.

Vaccine vectors

A series of different vaccine formulations has been developed incorporating recent insights into tumor rejection antigens, including peptide or protein plus adjuvant; *ex vivo*–loaded dendritic cells, recombinant viruses or bacteria; and DNA vaccines. The gene-based (usually DNA) vaccines by themselves have been found to be relatively weak. However, exceedingly strong cell-mediated immunity has been generated using a novel prime-boost strategy that involves initial priming with a DNA vaccine followed by boosting with pox virus or adenovirus vectors encoding similar heterologous antigens[25].

Enhancing the function of APCs

Efforts to enhance the efficacy of vaccines have focused on both the function of APCs such as dendritic cells and the activation of T cells. Dendritic cells mature through multiple stages, involving GM-CSF, flt3 ligand and IL-4 inducing cells, to an intermediate stage, where they are effective in the uptake of antigen but, if used, may cause induction of tolerance or of negative-regulatory T cells rather than an effective response[26,27]. Further differentiation of dendritic cells is induced by stimulation of toll-like receptors (such as lipopolysaccharide or unmethylated CpG DNA sequences) or members of the tumor necrosis factor (TNF) receptor family. Mature dendritic cells effectively present antigen to T cells associated with MHC molecules, in contrast to antigen-loaded immature dendritic cells, which can cause suppression of antigen-specific responses[26,27]. Another potential vaccine agent, interferon (IFN)-γ, could be used to generate mature dendritic cells for use in vaccines where a long memory $CD8^+$ response is desired. This suggestion emerges from the work of Tagaya and coworkers, who recently showed that IFN-γ and lipopolysaccharide induce coordinated expression of the IL-15 receptor α-subunit (IL-15Rα) and IL-15 by monocytes and dendritic cells[28]. They further showed that the cell-surface IL-15Rα generated by dendritic cells presents IL-15 in *trans* to neighboring cells, including $CD8^+$ cells, as a participant in the immunological synapse. The IL-15 presented by IL-15Rα interacts with IL-2R, IL-15Rβ and the common γ-chain expressed on interacting T cells, thereby facilitating the memory $CD8^+$ cell expression that is crucial for a sustained immune response to a vaccine[28].

Box 1 History of immunotherapy

1796	Jenner introduces vaccinia (cowpox) immunization to prevent subsequent smallpox infection.
1879–1886	Louis Pasteur introduces first laboratory-weakened infectious agent (chicken cholera bacterium) and shortly thereafter develops weakened rabies for active immunization.
1888	Emile Roux and Alexandre Yersin isolate toxin from diphtheria.
1890	Emil von Behring and Shibasabo Kitasato in Koch's laboratory find that injecting diphtheria toxin into animals produces a serum containing an antitoxin that provides passive anti-diphtheria immunity to people.
1900	Paul Ehrlich suggests that molecules that react with tumors could play a key role in cancer therapy, presaging antibody-mediated passive immunotherapy.
1954–1955	Jonas Salk and Albert Sabin introduce killed and live attenuated polio vaccines that soon lead to the elimination of poliomyelitis.
1965	IgG anti-D (anti-RH) is administered to prevent of RH immunization and thus prevent erythroblastosis fetalis; this is a translation of the basic insight that passive administration of a specific IgG antibody inhibits the active production of that antibody.
1975	George Köhler and Cesar Milstein develop hybridoma technology for monoclonal antibody generation.
1977	Smallpox is declared eradicated through vaccination.
1982	The first report of successful use of a monoclonal antibody to treat a human neoplasm (patient-specific anti-idiotype antibody to treat B-cell lymphoma) is reported.
1986	The first monoclonal antibody, muromonab-CD3 (Orthoclone OKT3), is approved by the FDA.
1986	The first humanized antibody is produced by replacing the complementarity regions in a human antibody with those of a mouse.
1986–2000	IL-2, IFN-α, IFN-β and IFN-γ are approved for use in the treatment of neoplasia, hepatitis and multiple sclerosis.
1988–1991	The methodology for isolating tumor antigens recognized by CTLs is introduced; the first human antigen from melanoma patients identified by CTLs is isolated.
1997	The first humanized monoclonal antibody (daclizumab, Zenapax) is approved by the FDA.
1997	The first monoclonal antibody (rituximab, Rituxan) for the treatment of malignancy is approved.
1998	An antibody to TNF-α (infliximab, Remicade), and p75 TNF receptor linked to the Fc of IgG1 (etanercept, Enbrel) are approved for use in the treatment of rheumatoid arthritis and Crohn disease.
2000	The first toxin-linked monoclonal antibody (gemtuzumab ozogamicin Mylotarg) is approved by the FDA.
2002	The first radionuclide-linked monoclonal antibody (ibritumomab tiuxetan, Zevalin) is approved by the FDA.

One approach to immunotherapy involves incorporating into the vaccines factors, such as GM-CSF, which induce dendritic cell differentiation[2,29,30]. In some approaches, the antigen is targeted to specific receptors on dendritic cells by using antigen–GM-CSF fusion proteins or immunoglobulin Fc region–antigen fusion proteins. In yet another approach, the antigen is complexed to members of the heat-shock protein family that enter the dendritic cell through CD91 (α2-macroglobulin receptor), an approach that substantially enhances the immunogenicity of the antigen by activating APCs and targeting them to the MHC processing pathway[6,31].

Fig. 1 Edward Jenner developed the first vaccine against smallpox in 1796

Enhancement of T-cell activation

Effective T-cell activation involves two sets of signals. One of these signals is mediated by the presentation of antigenic peptides, bound to MHC molecules, to the T-cell receptor; the other is mediated by the interaction of co-stimulatory molecules expressed on APCs with counter-receptors expressed on T cells. Many of the different antigens used in immunotherapy have very low affinities for their MHC molecules. Therefore, immunotherapies have been enhanced by the modification of the antigenic peptide by epitope engineering. This involves altering the antigen to increase its affinity for MHC molecules by taking advantage of known sequence motifs for peptide binding to the anchor residues that are involved in MHC binding[2,4,32,33]. In one strategy, a combinatorial library is used to screen sequences for improved MHC binding[34]. A complementary epitope enhancement approach is to increase the affinity of the peptide-MHC complex to the TCR[35].

A second category of immunotherapeutic augmentation focuses on the positive signals to T cells delivered by a large number of co-stimulatory molecules of the B7 and TNF families (such as CD40 ligand) expressed on the surface of APCs. This approach, which focuses on the expression of B7 (CD80) on APCs, is based on the view that stimulation is primarily mediated by APCs rather than by the tumor cells themselves. In addition, engagement of CD137 (4-IBB) expressed on dendritic cells, monocytes, natural killer (NK) cells and T cells induces cytotoxic T lymphocyte (CTL) immunity to tumors previously considered non-immunogenic[36].

Cytokines in immunotherapy

Hormones, which are generated at one site and destined to interact with receptors expressed on distant cells, have long been important therapeutic agents. In contrast, there has been less widespread use of the cytokines normally generated by the immune system for cell-to-cell communication. This may reflect the fact that such cytokines are normally dedicated to act in a very localized microenvironment as autocrine or paracrine factors at the site of an immunological synapse. Nevertheless, there are a number of such agents that are approved for clinical use in immunotherapy[37,38]. IFN-γ is used in osteopetrosis and chronic granulomatous disease, and IFN-β preparations are approved for multiple sclerosis. IFN-α is used in the treatment of hairy cell leukemia, malignant melanoma, follicular lymphoma, AIDS-related Kaposi sarcoma, and hepatitis B and C.

Another application of T-cell co-stimulatory cytokines involves incorporation of their genes into viral vaccines. GM-CSF, which acts on dendritic cells, provides the broadest range of T-cell responses, including Th1, Th2 and CTL[2]. Furthermore, incorporation of the gene encoding IL-12 into DNA vaccines yielded antigen-specific responses that were predominantly Th1, whereas inclusion of IL-4 or IL-10 induced a Th2 response[2,39]. IL-2 has received approval from the US Food and Drug Administration (FDA) for use in the treatment of metastatic renal cancer and malignant melanoma, where it induced a durable complete response in 5–10% of patients[37,38]. There are, however, limitations in the use of IL-2. In terms of the immune response, in addition to its role in the initial activation of T and NK cells, IL-2 has a critical role in the maintenance of peripheral tolerance[40]. In terms of this unique function, IL-2 has a central role in activation-induced cell death (AICD), a process that leads to the elimination of self-reactive T cells[40]. As a result of this pivotal role in AICD, the T cells generated in response to tumor vaccines containing IL-2 may interpret the tumor cells as self and the tumor-reactive T cells may be killed by AICD-induced apoptosis. Furthermore, IL-2 maintains $CD4^+$ $CD25^+$ negative regulatory T cells and has been reported to terminate $CD8^+$ memory T-cell persistence[41]. In parallel with IL-2, IL-15 is very effective in the activation of T, NK and NK-T cells[42]. In contrast to IL-2, IL-15 manifests anti-apoptotic actions, inhibits IL-2–mediated AICD and stimulates the persistence of $CD8^+$ memory cells[42–45]. In light of these valuable characteristics, IL-15 may be superior to IL-2 in the treatment of cancer and especially as a component of vaccines where a prolonged immune response is desirable[42,45].

Box 2 Progress in the development of prophylactic and therapeutic vaccines for cancer

- Tumor rejection antigens are identified by defining tumor-associated antigens recognized by host antibodies (SEREX technique), as well as by screening libraries from tumor tissues using tumor-reactive T cells from cancer patients or by MHC-bound peptide elution followed by mass spectrometric analysis.
- The function of APCs is enhanced by inducing the maturation of dendritic cells using agents such as GM-CSF, IL-4, TNF-α, CpG and CD40L. *Ex vivo*–loaded dendritic cells are used with antigens or DNA vaccine priming, followed by attenuated viruses as boosters or heat shock proteins to enhance vaccine potency.
- Signal I (MHC-antigen-TCR interaction) is augmented by epitope enhancement of the antigenic peptide to increase its MHC and TCR binding.
- Signal II (CD80; CD86 interaction with CD28) is augmented by incorporating a B7 family member into recombinant DNA or viral vectors used for immunization, or by use of the CD40 ligand or antibodies against CD137.
- Cytokines (such as GM-CSF, IL-2, IL-12 or IL-15) or the genes encoding them are introduced into vaccine preparations.
- Surrogate assays of vaccine efficacy are developed, including cytotoxicity, tetramer binding assays, and ELISPOT (enzyme-linked immunospot) to measure cytokine secretion.

Mechanisms that impede effective immunotherapy

There are a number of impediments to the effective immunotherapy of cancer that may limit successful treatment to individuals with minimal disease or may yield only transient tumor responses. Some of these impediments are tumor-cell associated, including the loss of class I MHC expression, failure to maintain the co-stimulatory B7 molecules, and the production by tumor cells of factors such as TGF-β or soluble cytokine or receptor mimics that inhibit effective immune responses[2,4]. However, the major impediments to effective immunotherapy are a series of negative immunoregulatory safety mechanisms that are normally dedicated to preventing self-reactive destructive immune responses that lead to autoimmune disease (Box 3). Among the best studied of these 'brakes' on the immune system is cytotoxic T-lymphocyte antigen-4 (CTLA-4)[46], a negative co-stimulatory molecule. The interaction of B7 family members with the CD28 co-stimulatory receptor is pivotal in the initiation of T-cell immune responses. However, the expression of CTLA-4, a second receptor that has a much higher affinity for B7, is induced after T-cell activation. CTLA-4 inhibits T-cell activation and IL-2 production[46]. Allison and coworkers showed that antibody-mediated blockade of CTLA-4 enhanced antitumoral immunity to a GM-CSF transduced vaccine, which in turn led to the regression of established transplanted syngeneic tumors[46].

As noted above, another negative regulatory control in the immune system, dedicated to the maintenance of self-tolerance, is AICD. One approach to avoiding this termination of a desired immune response involves the use of IL-15 instead of IL-2 as a component of vaccines[42].

A third system is mediated by $CD4^+$ $CD25^+$ negative-regulatory T cells that impede effective immune responses to tumor antigens. Different monoclonal antibodies directed toward CD25 have manifested different effects on such cells. The humanized monoclonal antibody directed to CD25 expressed on some human leukemic cells was effective in the therapy of select patients with CD25-expressing adult T-cell leukemia, uveitis and multiple sclerosis. When this antibody was administered for one to four years to patients with autoimmune disorders, it did not lead to a reduction in the number of circulating $CD4^+$ $CD25^+$ negative-regulatory cells. In contrast, the administration of the mouse CD25–specific monoclonal antibody PC61 to mice led to the depletion of $CD4^+$ $CD25^+$ regulatory T cells that permitted effective immune responses to certain syngeneic tumors; this resulted in the induction of CTL and NK cell cytotoxicity that in turn led to tumor rejection[47–49].

Yet another mode of inhibition of immunosurveillance is mediated by the $CD4^+$ NK T-cell production of IL-13, which in turn induces the expression of TGF-β that inhibits the antitumor cytotoxicity mediated by $CD8^+$ CTLs[50]. Blockade of this negative regulatory pathway by inhibiting IL-13 action using an IL-13 receptor–immunoglobulin fusion protein enhanced antitumor responses and potentiated the efficacy of vaccines[50]. In summary, the development of effective immunotherapeutic and immunopreventive vaccines for chronic diseases will require the identification of appropriate tumor-rejection antigens, the optimization of peptide, APC and T-cells interactions, and the blockade of suppressive negative regulatory mechanisms that impede immunotherapeutic efforts.

Passive immunotherapy

In 1888, Emil Roux and Alexandre Yerson isolated the toxin from the diphtheria bacterium. This provided the scientific basis for the work of Emil von Behring and Shibasaburo Kitasato in Robert Koch's laboratory. They injected small doses of the diphtheria toxin into animals to yield serum containing antibodies (antitoxin) that on administration to patients provided passive immunity to treat diphtheria.

Monoclonal antibodies as passive immunotherapeutics

The development of monoclonal antibodies by Köhler and Milstein captured the imagination of the medical community in 1975 (ref. 7). Monoclonal antibodies, however, are just beginning to fulfill the great promise for immunotherapy inherent in their specificity, which permits their selective binding to abnormal cells[11]. Despite wide-ranging efforts, the dream of a 'magic bullet' of antibody therapy prevailing since the time of Ehrlich has proven elusive[12]. However, Levy and coworkers induced remissions in select patients with B-cell lymphoma, using immunoglobulin-specific idiotypic monoclonal antibodies[21]. For a long time, only a single monoclonal antibody, muromonab-CD3 (Orthoclone or OKT3), was licensed by the FDA[51]. A number of factors underlie the low therapeutic efficacy observed. Unmodified mouse monoclonal antibodies are immunogenic to humans, have short *in vivo* survival, and generally do not kill target cells efficiently

because they do not fix human complement or elicit antibody-dependent cellular cytotoxicity (ADCC) with human mononuclear cells. Finally, in most cases the antibodies were not directed against a vital cell-surface structure such as a receptor for a growth factor that would be required for tumor cell survival and proliferation. To circumvent these problems, researchers have developed human as well as humanized antibodies (see Box 4)[8,52]. In addition, cell-surface antigenic targets, especially receptors for cytokines, have been found to provide more effective monoclonal antibody action[9]. Furthermore, cytotoxic action of monoclonal antibodies has been augmented by arming them with toxins or radionuclides. With these advances, 11 monoclonal antibodies have received FDA approval (see Box 5). At least 400 more monoclonal antibodies are in clinical trials[11].

The early successes involved monoclonal antibodies used to prevent organ allograft rejection. The initially approved monoclonal antibody muromonab-CD3 (Orthoclone or OKT3) targeted the CD3 element of the T-cell antigen receptor complex[51]. Subsequently approved antibodies include basiliximab (Simulect), a chimeric antibody, and the humanized anti-Tac antibody daclizumab (Zenapax), the first humanized antibody approved by the FDA. Both are directed toward the IL-2Rα subunit (CD25) and interfere with its interaction with IL-2. The humanized monoclonal antibodies that emerged from the work of Winter and coworkers and were modified by Queen and coworkers retain only the 5–10% of the mouse component that is involved in antigen antibody interaction[8,52]. Such humanized monoclonal antibodies show drastically reduced immunogenicity, improved pharmacokinetics and ADCC with human mononuclear cells. A recent clinical effort has focused on blockade of TNF-α for use in the immunotherapy of such autoimmune disorders as rheumatoid arthritis and inflammatory bowel disease[53]. The two licensed biological agents that target TNF-α include the chimeric monoclonal anti-TNF-α infliximab (Remicade) and an engineered p75 TNF receptor (TNF-R) dimer linked to the Fc portion of IgG1, etanercept (Enbrel). The scientific basis for this strategy is the demonstration that TNF-α is at the apex of a pyramid of inflammatory factors so that its inhibition leads to the simultaneous inhibition of the downstream inflammatory agents IL-1β, IL-6 and select inflammatory chemokines[53]. Both agents that inhibit binding of TNF-α to its receptor result in rapid improvement in symptoms and signs of rheumatoid arthritis and inflammatory bowel disease. However, a limitation in TNF-α as a target for immunotherapy is that it is not involved in the regulation of immunological memory[54]. Thus, on withdrawal of such therapy there is a high likelihood of recurrence of the clinical disorders. We proposed targeting IL-15 for these diseases because it is also involved in the inflammatory cascade acting as a major stimulus for TNF-α synthesis[42]. Furthermore, abnormalities of IL-15 have been demonstrated in autoimmune diseases. However, in contrast to TNF-α, IL-15 is required for the proliferation and maintenance of memory $CD8^+$ cells[42–44]. Thus, the interruption of IL-15 action may reduce the persistence of memory and effector $CD8^+$ self-reactive lymphocytes. Proposed IL-15–directed approaches have included antibodies to IL-15, the use of a fusion protein involving the IL-15Rα subunit linked to the Fc portion of IgG, and mutant forms of IL-15 that have antagonistic action[42]. Our own approach involves the administration of an antibody against the IL-2 and IL-15Rβ receptor, shared by IL-2 and IL-15, that blocks all IL-15 action[42]. It should be noted that these IL-15–directed approaches that interfere with the persistence of memory T cells carry the risk of increasing the rate of development of recurrent, chronic infectious diseases such as tuberculosis.

There have also been important advances in the use of monoclonal antibodies directed toward cancer cells, with the monoclonal antibody rituximab (Rituxan) being the first ap-

Box 3 Blockade of negative immunoregulatory mechanisms that impede immunotherapy

Host negative immunoregulatory mechanism	Potential intervention to release 'brake' on the immune response
Signals mediated by the negative T-cell co-stimulatory molecule CTLA4, after interaction with the B7 family on APCs,deliver inhibitory signals that terminate T-cell activation.	Antibody-mediated blockade of CTLA-4 enhances antitumor immunity induced by vaccines, which leads to the re gression of established tumors.
IL-2–mediated AICD yields self-tolerance, which leads to apoptotic death of tumor-specific T-cells.	Use of IL-15, which inhibits AICD, instead of IL-2 in cancer therapy and as a component of vaccines.
$CD4^+CD25^+$ negative regulatory (suppressor) T cells inhibit antitumor immune responses.	CD25-directed antibody therapy to deplete suppressor cells.
$CD4^+$ NK T-cell generation of IL-13 that indirectly (through the action of TGF-β) inhibits $CD8^+$ cell mediated antitumor responses.	Administration of IL-13Rγ2 Fc or IL-13–specific monoclonal antibody to eliminate IL-13 effects.
C1S/SOCS (cytokine-inducible SH_2-containing protein/ suppressor of cytokine signaling) family, and phosphatases (such as SHP-1) terminate cytokine signaling through the Jak-STAT pathway. Inhibition of immune responses by prostaglandin E2 and nitric oxide synthase.	Small molecule targeting of these negative regulators of T-cell receptor and cytokine signaling.

Box 4 Progress in the development of monoclonal antibodies

Modification	Advantage	Disadvantage
Humanization of mouse monoclonal antibodies. Production of antibodies in humanized mice.	Reduced immunogenicity, improved pharmacokinetics and increase in effector (such as ADCC) action.	High cost of production. Unarmed antibodies have relatively low cytotoxicity for target cells.
New approaches to the production of monoclonal antibodies (such as by phage display or production in plants).	Ability to produce antibodies in large quantities, against targets that may be difficult when using intact animals.	Low-affinity antibodies may initially be produced by phage display.
Genetic engineering to alter affinity or survival of monoclonal antibody.	CDR can be altered to increase affinity of binding. Immunoglobulin CH_2 domain can be deleted or $Fab_1F(ab')_2$ or Fv element can be used to reduce *in vivo* survival of a radionuclide-armed antibody.	
Alterations to Fc region to reduce binding to inhibitory Fc-γ RIIB and to increase binding to activation receptors.	Increase ADCC-mediated target cell killing by increasing engagement of Fc-γ RIII receptors on host mononuclear cells.	
Arming of monoclonal antibodies (such as CD22-, CD25- and CD33-specific)with toxins (such as *Pseudomonas*, exotoxin, ricin or diphtheria toxin).	Increased cytotoxicity when compared with unmodified antibody.	Highly toxic immunogenicity limits number of courses of therapy.
Arming of intact monoclonal antibodies with radionuclides.	Increased cytotoxicity.	Bone marrow toxicity due to long *in vivo* survival of intact monoclonal.
Pre-targeting of radionuclides with nonradioactive Fv-streptavidin-fusion protein, followed by the radionuclide-linked to biotin chelate.	Marked increase in tumor-to normal-tissue radionuclide ratio with increase in tumor dose possible, compared with intact antibody.	Immunogenicity of streptavidin limits number of courses of therapy.

proved to treat malignancy. Using the CD20-specific antibody rituximab (Rituxan) in individuals with relapsed low-grade non-Hodgkin lymphoma, there was an overall response rate of 57% for the group of patients receiving eight weekly infusions, with 14% of these patients manifesting complete and 43% partial responses[55,56]. In addition, trastuzumab (Herceptin), a humanized antibody against the HER2/neu tyrosine kinase receptor provided an overall response rate of 15% in 222 patients with high expression of HER2/neu associated with breast cancer[57]. Both rituximab and Herceptin have been shown to improve the overall survival of appropriate patients when added to standard chemotherapy in randomized trials[57–59]. Furthermore, we have observed remissions in one-third of patients with HTLV-1–associated adult T-cell leukemia receiving monoclonal antibody therapy directed against IL-2Rα[60]. A number of strategies have been used to increase the therapeutic impact of these antibodies, including the use of genetic engineering to alter the antibody affinity for the target ligand or to modify the constant region involved in Fc receptor binding. Clynes, Ravetch and coworkers showed that although many mechanisms have been proposed to account for the antitumor activities of therapeutic antibodies (such as blockade of signaling pathways, activation of apoptosis and cytokine deprivation–mediated cell death), engagement of Fc-γ receptors on effector cells is a dominant component of their *in vivo* antitumor activity[61,62]. Using Fc-γ–deficient mice that cannot express Fc-γ RIII, the stimulatory Fc receptor, they demonstrated that the efficacy of the therapeutic agents trastuzumab and rituximab in mouse xenograft models requires engagement by the antibody of this receptor. Furthermore, they showed that the inhibitory Fc-γ RIIB receptor is a potent negative regulator of ADCC *in vivo*[62]. These studies provide the scientific basis for genetic engineering of monoclonal antibodies to improve binding to the Fc-γ RIII stimulatory receptor while reducing binding to the Fc-γ RII inhibitory Fc receptor. A number of companies have initiated efforts to achieve this goal, despite the fact that this represents a challenge given the high degree of sequence identity between Fc-γ RII and Fc-γ RIII.

Antibodies armed with toxins and radionuclides

A major limitation to the use of monoclonal antibodies in the treatment of cancer is that most are poor cytocidal agents. To address this issue, monoclonal antibodies are being linked to a cytocidal agent, such as a toxin or radionuclide, which is then targeted to the tumor cell by the antibody. The arming of antibodies with toxins has been stimulated by the approval of the first immunotoxin-armed antibody gemtuzumab ozogamicin (Mylotarg), which links the toxin calicheamicin to a CD33-specific antibody for use in the treatment of myelogenous leukemia. In addition, protein-toxin conjugates with a truncated *Pseudomonas* endotoxin genetically linked to a

CD25- or CD22-specific antibody have induced remissions in patients with hairy cell leukemia[63,64].

Radioimmunoconjugates

Oncotoxins are immunogenic and manifest toxicity to normal tissues, and thus provide only a narrow therapeutic window before the development of antitoxin antibodies. Radiolabeled monoclonal antibodies have been developed as alternative cytotoxic immunoconjugates[65–71]. A number of components must be considered in designing an optimal systemic radioimmunotherapeutic agent, including (i) selection of the monoclonal antibody and thus the antigenic target, (ii) choice of the delivery system used to target the radionuclide to the tumor cell, and (iii) choice of the radionuclide. CD20, the major target used in the therapy of B-cell leukemia and lymphomas, has an expression limited to B cells. The unmodified antibody rituximab alone or armed with ^{90}Y (ibritumomab tiuxetan; Zevalin) has been approved for the treatment of non-Hodgkin lymphoma[65]. The IL-2Rα subunit (CD25), identified by the anti-Tac monoclonal antibody, has also been used as a target for the treatment of T-cell leukemias and lymphomas[66]. The scientific basis for this choice is that IL-2Rα is not expressed by most resting cells, whereas it is expressed by the abnormal cells in certain forms of lymphoid neoplasia. A second issue in designing an optimal radioimmunotherapeutic reagent is the choice of the method used to deliver the radionuclide to the tumor cell. Most clinical trials use intact monoclonal antibodies to deliver the radionuclide. Although this approach has provided meaningful efficacy, only modest tumor-to-normal-tissue radionuclide ratios are achieved. In addition, the long serum half-life of intact monoclonal antibodies prolongs radiation exposure to normal organs, which limits the radiation dose that can be safely administered. To circumvent these obstacles, various approaches, including pre-targeting strategies that separate the antibody targeting from the delivery of the radionuclide, have been developed initially by Axworthy and coworkers and then validated in the studies of others[69–71]. In recent efforts, streptavidin was initially targeted to the IL-2Rα receptor selectively expressed on the tumor-cell surface using an anti-Tac (CD25) single chain Fv-Streptavidin–fusion protein (scFvSA)[71]. This was followed by administration of chelated biotin armed with a radionuclide. This low-molecular-weight cytotoxic molecule reached the tumor rapidly, where it was captured by the localized scFvSA or alternatively is eliminated in the urine. Using this pre-targeting approach, large quantities of radioactivity were delivered to the tumor with a dramatic increase in both the tumor-to-normal-tissue ratio of radioactivity delivered and the efficacy achieved. The third component of an optimal radioimmunotherapeutic regimen to consider is the nature of the radionuclide used. Most published clinical studies used the β-emitting radionuclides ^{90}Y or ^{131}I. Such β-emitting radionuclides depend on crossfire for their action on large tumor masses. However, as the tumor mass decreases, the benefit of the crossfire effect also decreases. With various small tumors including leukemias, the therapeutic effect of high-energy β-emitting radionuclides is limited because they yield a high dose of irradiation outside of the tumor volume as a result of the long path of the β-irradiation. For such forms of malignancy, the development of pre-targeting approaches may focus on α-emitting radionuclides that could be the most effective agents for killing tumor cells without damaging adjacent normal tissues.

Summary

After decades of disappointment, active immunotherapy with vaccines, as well as passive immunotherapy using unmodified and armed monoclonal antibodies, are emerging as use-

Box 5 Monoclonal antibodies approved by the US Food and Drug Administration

Product	Type	Target of action	Condition	Approved
Muronomab-CD3 (Orthoclone OKT3)	Mouse	CD3 antigen on T cells	Transplant allograft rejection	1986
Abciximac (ReoPro)	Chimeric	Glycoproteins IIb and IIIa on activated lymphocytes	Cardiovascular disease	1994
Daclizumab (Zenapax)	Humanized	CD25 (IL-2Rα, Tac) on activated lymphocytes	Transplant allograft rejection	1997
Rituximab (Rituxan)	Chimeric	CD20 on B lymphocytes	Non-Hodgkin lymphoma	1997
Basiliximab (Simulect)	Chimeric	CD25 (IL-2Rα) on activated lymphocytes	Transplant allograft rejection	1998
Palivizumab (Synagis)	Humanized	F protein on respiratory syncytial virus	Respiratory syncytial virus	1998
Infliximab (Remicade)	Chimeric	TNF-α	Rheumatoid arthritis, Crohn disease	1998
Trastuxumab (Herceptin)	Humanized	HER2 oncoprotein	Metastatic breast cancer	1998
Gemtuzumab ozogamicin (Mylotarg)	Humanized, toxin-linked	CD33 on leukemic blasts	Acute myelogenous leukemia	2000
Alemzutumab (Campath 1H)	Humanized	CD52 on B, T and NK cells and monocytes	Chronic lymphocytic leukemia	2001
Ibritumomab tiuxetan (Zevalin)	Chimeric, radionuclide-linked	CD20 on B lymphocytes	Non-Hodgkin lymphoma	2002

ful immunotherapeutic strategies. Many hurdles remain before the prevention or cure of chronic infectious diseases or cancer with immunotherapy becomes routine. Nevertheless, we are making progress toward fulfilling the vision of Paul Ehrlich, who stated in his Croonian lecture, "On Immunity with Special Reference to Cell Life," to the Royal Society of London a century ago[12]:

"It is hoped that immunizations such as these, which are of great theoretic interest, may come to be available for clinical application attacking epithelial new formations, particularly carcinoma by means of specific anti-epithelial sera…I trust, my lords and gentlemen, that we no longer find ourselves lost on a boundless sea, but that we have already caught a distinct glimpse of the land where we hope, nay, which we expect, will yield rich treasures for biology and therapeutics."

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