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Design of effective immunotherapy for human autoimmunity

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A better understanding of the molecules involved in immune responses has identified many potential targets for the treatment of autoimmune diseases. But although successful therapies have been found for immune disorders in animal studies, few have passed the much harder test of treating human diseases. So far, non-antigen-specific approaches, such as the blocking of tumour-necrosis factor, are achieving some success but the same is not true for antigen-specific approaches. Future therapies will probably include both non-antigen-specific strategies that target cytokines (cell–cell signalling molecules) or block the molecules that stimulate immune responses, and antigen-specific therapies that induce tolerance to self antigens.

Immunotherapy is a type of treatment that uses immunological tools, such as monoclonal antibodies, receptor–immunoglobulin fusion proteins, vaccines and immune cells. Such therapeutic options have only been available in the past 10 to 15 years, owing to major advances in medical science and technology, but are now increasingly being used to tackle a wide spectrum of human diseases. The application of immunotherapy to autoimmune diseases is broadening our understanding of the human immune response, with responses to treatment providing unique insights into pathological mechanisms. The availability of effective immunosuppressive drugs[1] to ameliorate the immune-mediated rejection of transplants contrasts sharply with the paucity of drugs that successfully treat autoimmune diseases. This implies that whereas a transplant is a classic acute challenge to an otherwise normal immune system, chronic autoimmune diseases are somehow different.

The failure of most immunological approaches that are effective in animal models[2,3] to modulate autoimmune disease in humans suggests that we do not understand many of the principles behind the pathogenic mechanisms of these diseases. We remain ignorant of what drives the chronicity of these conditions, which can last for decades, and of how we can normalize the immune and pro-inflammatory responses once they commence. The rate-limiting steps of the early immune response (such as the presentation of antigen by dendritic cells, the expansion of $CD4^+$ helper T-cell populations and the induction of costimulatory-molecule expression) may not be rate limiting or critical for the chronic phase of the disease and the resultant tissue destruction, which often occur years after onset.

Human transplants, which often undergo chronic rejection[1] despite continuous immunosuppressive therapy and early success, have confirmed our lack of understanding of chronicity. Furthermore, results in acute animal models of autoimmunity are often not predictive for the treatment of chronic human immune disorders[2–4]. Because we do not understand the differences between the chronic and acute response, we cannot be sure which, if any, animal models of disease provide good reflections of the key processes that occur in human disease. A further complication for the transition from animal to human studies is the necessary preoccupation with safety in human immunotherapy, a relatively ignored issue in animal models.

Here, we highlight recent successes in immunotherapy, which is now benefiting almost a million patients with chronic diseases, such as rheumatoid arthritis and Crohn's disease, that are unresponsive to other treatments. We contrast the effectiveness of therapies aimed at inhibiting the non-antigen-specific pathways, such as cytokine and cell-trafficking pathways (components of innate immunity), with the comparative lack of success of therapies that interfere with the more complex and flexible features of antigen-specific adaptive immunity.

Targets for immunotherapy

The treatment of human autoimmune diseases often occurs years after the onset of the pathogenic process, and despite our increasing knowledge of the cellular and molecular processes involved in immunity, the most effective targets for immunotherapy in the chronic phase of the disease are not obvious. Targeting various critical molecules involved in pathological pathways has led to the modulation of disease in animal models (Fig. 1). Components of the pathological cascade that have received most attention are: factors involved in lymphocyte homing to target tissues; enzymes that are critical for the penetration of blood vessels and the extracellular matrix by immune cells; cytokines that mediate pathology within the tissues; various cell types that mediate the damage at the site of the disease, as well as these cells' antigen-specific adaptive receptors, including the T-cell receptor (TCR) and immunoglobulin; and other toxic mediators, such as complement components and nitric oxide (Fig. 1).

A widespread misconception is that every step of the immune or pro-inflammatory process is a potential therapeutic target. Regrettably, this is not the case. Because most therapeutics only have a partial inhibitory effect, only those molecules that are in short supply (and thus rate-limiting) are likely to be useful targets. Therefore, therapy that specifically targets most of the steps (which are non-rate-limiting) in the immune or pro-inflammatory process yields little benefit in ongoing (late, active) autoimmune disease in humans. So far, only therapies that target two rate-limiting steps — the cytokine tumour-necrosis factor (TNF; ref. 5) and the molecule involved in lymphocyte homing, $\alpha_4\beta_1$ integrin[6] — have markedly ameliorated autoimmune disease progression; for example, in rheumatoid arthritis, inflamma-

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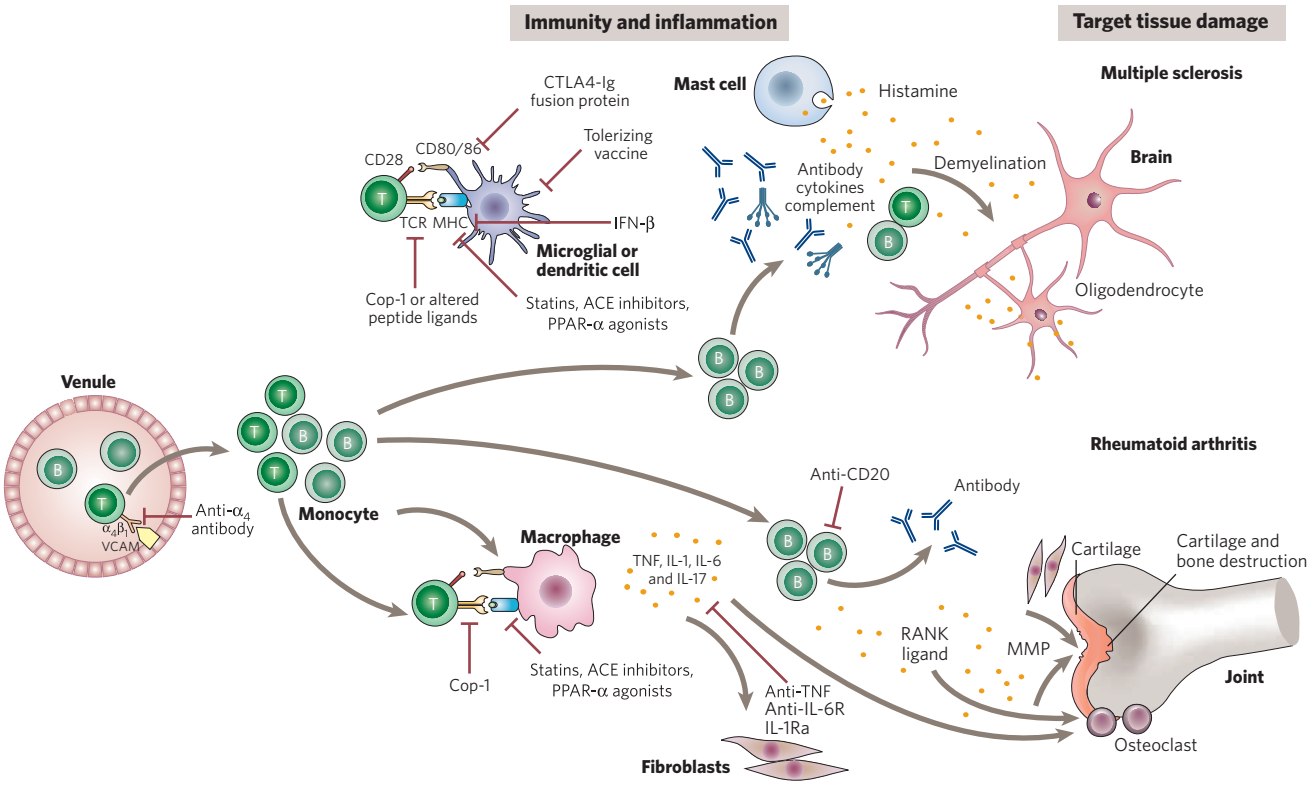

Figure 1 | Pathogenesis of multiple sclerosis and rheumatoid arthritis. Activated T cells express $\alpha_4\beta_1$-integrin, which binds to vascular cellular adhesion molecule (VCAM) on the surface of venules in inflamed tissues. This interaction allows the T cells to pass through the endothelial wall and penetrate the extracellular matrix. In multiple sclerosis (upper panel), the T cells re-encounter the cognate CNS antigen presented by MHC class II molecules on either microglial or dendritic cells. This interaction can be inhibited by glatiramer acetate (Cop-1) or altered peptide ligands. In addition, statins, angiotensin-converting enzyme (ACE) inhibitors, and PPAR-α agonists can all downregulate the inducible expression of MHC class II molecules. Similarly, cytokines such as interferon-β (IFN-β) downregulate MHC class II molecules and interfere with diapedesis of cells (the movement of cells through the endothelial wall) by downregulating metalloproteases. CD28 and CD80/86 interactions can be blocked by the CTLA4-Ig fusion protein. Tolerizing vaccines promote tolerance processes which occur when the T cell/dendritic cell interaction is not optimal. B cells and mast cells are also recruited into the inflammatory infiltrate. Antibody plus complement can produce 'membrane attack' complexes, which can damage the oligodendrocytes and underlying axon. Osteopontin is expressed on the surface of oligodendroglial cells and neurons during active disease, and is pivotal in the disease progression. In rheumatoid arthritis, T cells and macrophages that have entered the synovium from inflamed venules produce cytokines, especially TNF, IL-1, IL-6 and IL-17, which mediate damage to the synovium. This damage can be blocked by anti-TNF antibody, IL-1 receptor antagonist (IL-1Ra), and anti-IL-6 receptor (IL-6R) antibody. RANK ligand is the main signal for activating osteoclasts in cartilage, which mediate bone destruction. Anti-TNF antibodies reduce the migration of lymphocytes from the blood to the synovium, and also prevent bone loss by blocking the destructive effects of IL-1, IL-6 and TNF. Anti-CD20 kills B cells but not plasma cells; fibroblasts make most of the tissue-destructive metalloproteinases (MMPs).

tory bowel disease, ankylosing spondylitis, psoriasis and multiple sclerosis.

These particular key molecules and the processes they control can be referred to as 'tipping points'[7]. In epidemiology, a tipping point is defined as the moment when epidemics qualitatively change, reach a critical mass and have major repercussions. This concept is valuable in autoimmune diseases because many cellular and molecular processes contribute to tipping the balance towards the disease state, and therefore are potential therapeutic targets. But although targeting these tipping points may provide significant benefit, in terms of treating autoimmune disease, blocking these critical physiological molecules could also negate their beneficial roles in generating protective immune responses, and therefore could lead to an increased risk of infection. For example, despite the enormous success in treating multiple sclerosis by blocking $\alpha_4\beta_1$ integrin, this treatment was recently voluntarily withdrawn because of the development of a fatal untreatable infection. So tipping points are physiological processes that are key to maintaining both health and disease.

The targeting of TNF (ref. 8) or $\alpha_4\beta_1$ (refs 6, 9, 10) has remarkable effects on several autoimmune diseases, including rheumatoid arthritis, inflammatory bowel disease, ankylosing spondylitis, psoriasis and multiple sclerosis. These molecules can therefore be considered as true tipping points in the pathophysiology of autoimmune disease. But

Box 1 | Using commonly used drugs to treat autoimmunity

Recently, familiar oral medications, such as statins and angiotensin blockers, widely used for other disease conditions such as hypercholesterolaemia, hypertriglyceridaemia, allergy and hypertension, have been shown to inhibit some of the biochemical reactions that occur in autoimmune inflammation (Fig. 1). These drugs have shown promise in pre-clinical models of autoimmunity, as well as in early-stage clinical trials[11]. Even if they are not optimal therapies on their own, they are clearly pointing towards key alternative pathways, and may prove to be effective when used in synergy with other approaches.

Interestingly, the statins, which block the activity of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and thus reduce levels of cholesterol, also inhibit the appearance of inducible MHC class II molecules[72]. The statins are remarkably potent in reversing disease in animal models, inducing shifts from the production of T_H1-type pro-inflammatory cytokines by autoaggressive T cells to T_H2-type cytokines[74]. Initial trials administering statins to multiple sclerosis and rheumatoid arthritis patients show moderate efficacy[71,75]. As with statins, peroxisome proliferator-activated receptor-α (PPAR-α) agonists — drugs used in type II diabetes — which regulate the activation of adipocytes and macrophages, also induce a shift in cytokine production from the T_H1 to T_H2 type[76]. Initial experiments suggest angiotensin blockers do the same[77]. The efficacy of the statins, PPAR-α agonists and angiotensin blockers may result from their ability to alter a number of pathological processes in the immune cascade.

because the benefit seen here is achieved by interfering with processes that are involved in both host defence and autoimmune pathology, the overall benefit:risk ratio is inherently difficult to predict.

In the pharmaceutical industry, drugs in only about 5% of the 'small-molecule' drug projects end up as approved therapeutics; most drop out because of problems, usually toxicity. Hence, existing drugs (which are relatively safe) with new potential uses present a wonderful opportunity. Recently, familiar oral medications, such as statins, which are widely used for other disease conditions, have been shown to be effective in animal models of autoimmunity and early-stage clinical trials in patients with multiple sclerosis and rheumatoid arthritis[11] (Box 1).

Non-antigen-specific approaches

T-cell populations and antigen-presenting cells

Despite preventing disease (such as arthritis and experimental autoimmune encephalomyelitis, EAE), to an impressive extent in animal models, anti-CD4-antibody therapy, with either lytic or non-lytic monoclonal antibodies, has not successfully treated human rheumatoid arthritis[12], psoriasis or multiple sclerosis[13]. However, the limited scope for experimentation in humans during clinical trials may mean that inappropriate antibodies or dose regimes have been used. Alternatively, failure to prevent disease might have been caused by the anti-CD4 antibody also inhibiting regulatory T cells that express CD4. By contrast, encouraging results have been reported from both animal models[14] and early clinical studies[15] using a mutated, less activating form of anti-CD3 antibody. The use of this antibody avoids the acute cytokine release — that causes a range of problems from malaise to hypotensive shock[16] — induced by non-mutated anti-CD3 antibody.

There is a growing consensus that antigen-presenting cells (APCs) are important rate-limiting cells for inducing immune responses[17]: a leading hypothesis is that inducible major histocompatibility complex (MHC) class II molecule expression is induced inappropriately on APCs at the site of autoimmune disease[18] (Fig. 1). Consistent with this, in many animal models of autoimmune disease, antibodies specific for MHC class II molecules reduce disease. But because the antibodies caused unexpected toxicity when tested in monkeys[19], this has not yet been tested in humans.

Effective antigen presentation and activation of T cells requires not only TCR recognition of MHC molecules complexed with a peptide, but also various ligand–receptor costimulatory interactions at the 'immune synapse' — the point of interaction between a T cell and an APC. Most important among these costimulatory interactions are CD28 molecules recognizing CD80 or CD86 molecules[11] (Fig. 2). Therapy using a cytotoxic T-lymphocyte antigen 4 (CTLA4)–immunoglobulin fusion protein, which blocks interactions with CD28, is effective in randomized, double-blind clinical trials in patients with psoriasis and rheumatoid arthritis[20], suggesting that even in late-stage disease, signals mediated by costimulatory molecules expressed by APCs are required. Blocking other molecules that are involved in activating the immune system may also be useful therapeutically. Unfortunately, despite promising results in experimental studies, the administration of an antibody specific for the T-cell-expressed costimulatory molecule CD40 ligand was toxic in humans, causing a number of deaths from thrombosis. The blocking of costimulatory molecules that are expressed only after antigen activation of T cells, such as OX40, may be efficacious and safer[21], as this would not block uninvolved T cells.

Regulatory T cells and B cells

Several regulatory subsets of T cells have been defined in recent years, and attention is now turning to their use for therapy. This is because defects in such regulatory subsets (in particular, the $CD4^+CD25^+$ regulatory T-cell subset) may be important in enabling autoimmune diseases to become established[22,23] (see review by Kronenberg and Rudensky in this issue, page 598).

Given the ubiquity of autoantibodies in autoimmune diseases, it was assumed that the antibody-producing cells — plasma cells and B cells — would be a good target for therapy. However, this assumption has only recently been confirmed: lytic anti-CD20 antibody (rituximab; Rituxan), which lyses B cells, effectively treated rheumatoid arthritis and systemic lupus erythematosus[24], although extensive comedication of subjects in these trials makes the data difficult to interpret.

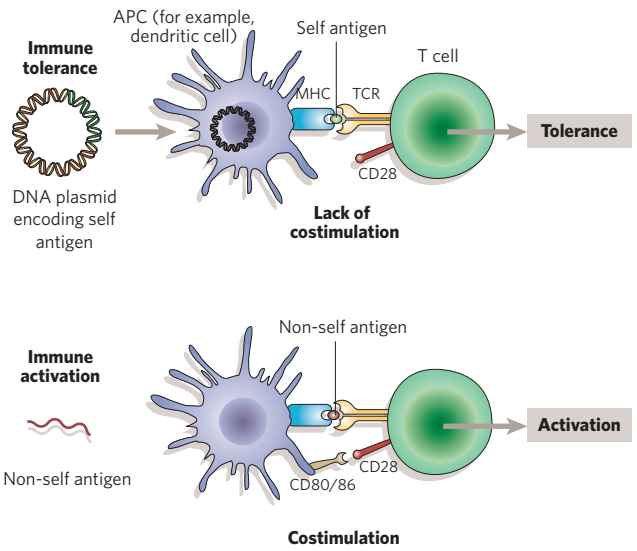

Figure 2 | Generating immune tolerance by using 'tolerizing' DNA vaccines. A DNA plasmid encoding a self antigen is transcribed and translated in a dendritic cell, but its expression does not stimulate the innate immune system enough to upregulate costimulatory molecules. A further reduction in costimulation is caused by the removal of CpG motifs in the plasmid. The presentation of self antigen by APCs without adequate costimulation leads to anergy or tolerance of T cells, because of the lack of interaction between CD28 with CD80 or CD86 (refs 72, 73). In contrast, conventional immunization, with a foreign antigen, leads to effective presentation of antigen in the MHC molecules with adequate costimulation, and leads to productive cytokine cascades and gene activation.

Cytokines

Cytokines are short-range protein mediators with a wide range of actions. They are important in all biological processes[25], including T-cell growth (IL (interleukin)-2, IL-4, IL-7, IL-15 and IL-21), inflammation (TNF, IL-1, IL-6 and IFN (interferon)-γ) as well as the inhibition of inflammation (IL-10, transforming growth factor-β (TGF-β) and IL-4). As extracellular molecules, they are accessible to 'biologicals' — protein therapeutics such as antibodies or soluble receptors.

The relative potency of cytokines that induce multiple biological effects is compatible with a rate-limiting, 'catalytic' role, and therefore they are potential therapeutic targets. A major problem in establishing which ones may be targets lies in the considerable overlap (redundancy) in their biological properties. Thus, IL-1, TNF, IL-6 and granulocyte–macrophage colony-stimulating factor (GM-CSF) have more than 80% overlap in function, when tested *in vitro*. So, which ones are likely to be therapeutic targets in which diseases? Insights into this problem have come both from *in vivo* experiments using animal models and from clinical studies[26].

In contrast to the limited success of treatment with cytokines (see below), blockade of cytokines is the success story of the current era of molecular therapy in autoimmunity, which is based on scientific analysis of disease mechanisms[8]. Research using joint tissue from patients with rheumatoid arthritis suggested the importance of TNF in the disease pathogenesis[27]. The existence of TNF-inhibiting biologicals (originally generated to treat sepsis syndrome) made it possible to perform a successful proof-of-principle clinical trial in 1992 (ref. 28) with the anti-TNF monoclonal antibody infliximab. This culminated

in the approval from 1998/1999 of a set of therapeutic biologicals: anti-TNF monoclonal antibodies (infliximab[29] and adalumimab[30]) and the TNF-receptor (TNFR) fusion protein (etanercept[31]; Enbrel). TNF blockade has demonstrated that biologicals can be used in the long term, and extensively: about a million patients have been treated with anti-TNF biologicals so far, and some for over seven years.

Much has been learnt from the use of anti-TNF biologicals; for example, the importance of finding the right therapeutic target. TNF is the body's fire alarm[5]. It initiates the defence response to local injury, recruits leukocytes, and initiates a whole series of events that are important in health and in many diseases. Hence its blockade is useful in treating many diseases. The mechanism-of-action studies (see Box 2) have provided several insights into the pathogenesis of targeted diseases[32], especially rheumatoid arthritis.

IL-6 is another useful target, with clinical-trial success in rheumatoid arthritis showing comparable efficacy to TNF blockade[33]. However, the clinical benefits of IL-6 blockade occur more slowly than with TNF blockade, as predicted from *in vitro* studies that revealed a TNF-dependent cytokine cascade[27,34], where TNF drives the production of multiple pro-inflammatory cytokines. Success has also come from IL-1 blockade using the IL-1-receptor antagonist anakinra, which is approved for the treatment of rheumatoid arthritis[35]. And promising results have been seen in the treatment of rheumatoid arthritis with anti-IL-15 antibody[36]. High mobility group 1 (HMGB1), a stimulator of inflammatory responses, is another promising target for arthritis and sepsis[37]. Finally, blocking the receptor activator of nuclear factor NF-κB ligand (RANKL), the main activator of osteoclasts, is a promising approach for reducing bone destruction, such as that seen in rheumatoid arthritis[38].

Cell recruitment: chemokines and adhesion molecules

The small-protein chemotactic cytokines (chemokines) have several properties that make them favoured targets in the pharmaceutical industry[39]: they are extracellular, and so accessible to biologicals; and they bind to seven-transmembrane receptors that can be blocked by small-molecular-mass chemicals. Most importantly, chemokines are mediators of cell migration. Because chronic inflammatory diseases depend on the recruitment of inflammatory cells to the inflamed site, any approach that reduces the number of inflammatory cells in the site of disease may be of benefit, be it through chemokine or adhesion-molecule blockade. However, like cytokines, there are numerous chemokines (more than 40) with redundant properties, so it is not clear which ones are the most relevant in which disease.

Immune surveillance is accomplished by highly mobile leukocytes that are primed to fight microbes anywhere in our bodies. Organ-specific autoimmunity may result when autoreactive lymphocytes enter an inflamed site, initiating multiple events[8,18]. Lymphocyte migration depends on highly specific 'adhesion' molecules expressed by T cells that bind to receptors induced on endothelial cells[40]. These adhesion molecules and their receptors have domains in the extracellular space, and so can be targeted with monoclonal antibodies. Because the key homing molecules — integrins and selectins — display a high degree of diversity, a particular integrin molecule or selectin molecule is critical for entry to a particular anatomical site, and blocking that molecule might abolish pathological homing to that site, leaving lymphocytes free to move elsewhere.

Initial studies in animal models of multiple sclerosis (EAE) indicated that the critical homing molecule to the inflamed central nervous system (CNS) is $\alpha_4\beta_1$ integrin[9]: anti-$\alpha_4\beta_1$ antibody blocked the entry of lymphocytes into the brain and abrogated the clinical paralysis associated with EAE. This approach also proved successful in patients with multiple sclerosis: a phase III trial of a humanized $\alpha_4\beta_1$-specific monoclonal antibody natalizumab (Tysabri) reduced clinical relapses by 66% over the next year, leading to Food and Drug Administration (FDA) approval of the drug[6]. Encouraging results were seen with the same antibody in the treatment of inflammatory bowel disease[10].

However, the blockade of $\alpha_4\beta_1$ integrin is not specific. It interferes with lymphocyte homing in general, and therefore raises the risk of opportunistic infections[11]. Recently, sales of natalizumab were withdrawn, after two patients taking it in combination with IFN-β1a (Avonex) developed progressive fatal multifocal leukoencephalopathy, an untreatable viral infection (http://www.fda.gov/cder/drug/infopage/natalizumab/default.htm). Blocking lymphocyte mobility with these two drugs, and blocking lymphocyte entry to the brain, may have caused this unusual infection, caused by the ubiquitous JC virus, the activation of which is most commonly seen in severely immunocompromised individuals.

Box 2 | Anti-TNF therapy of rheumatoid arthritis

Mechanism of action
- Reduction in pro-inflammatory cytokine cascade, including reduction of IL-6, IL-1, GM-CSF and vascular endothelial growth factor (VEGF).
- Reduction in leukocyte trafficking owing to decreased expression of adhesion molecules and chemokines.
- Reduction in tissue-destructive enzymes, such as matrix metalloproteinases (MMPs), but levels of tissue inhibitor of MMPs are maintained.
- Reduction in angiogenesis through reduced VEGF production.
- Normalization of abnormal haematology: haemoglobin restored, platelets and fibrinogen reduced.

Clinical benefits
- Reduction of symptoms including pain, stiffness and lethargy.
- Reduction in signs of active disease including tenderness and joint swelling.
- Reduction in cartilage and bone damage.
- Induction of tissue repair.

Potential side effects
- Increased risk of infection due to reduced cytokine, for example increased risk of TB and pneumonia.
- Increased levels of antibodies to double-stranded DNA; rare cases of drug-induced lupus can occur.
- Increased risk of lymphomas (not proven).

Differences between TNF-blocking drugs
- Etanercept blocks TNF and lymphotoxin a (LTa).
- Infliximab and adalumimab, but not etanercept, are active in Crohn's disease.
- Difference most likely to be due to different dosing regimes.
- Alleged differences in cytotoxicity/apoptosis are controversial.

Antigen-specific approaches

The adaptive autoimmune response becomes more complex as disease progresses, owing to the generation of T-cell reactivity and antibodies to other local molecules — a concept known as epitope spreading[41]. Thus, in the chronic stage of the disease, the adaptive immune response targets several different molecules at the anatomical site of the disease.

In the 1970s, a random copolymer of the amino acids glutamate, tyrosine, alanine and lysine (copolymer 1 or Cop-1), now termed glatiramer acetate or copaxone, was designed to mimic the composition of myelin basic protein (MBP) — a major target of autoimmune responses in multiple sclerosis. The administration of glatiramer acetate ameliorated EAE, and is now an approved drug for multiple sclerosis[42]: daily injection of glatiramer acetate reduces disease relapse by 30%, and induces a T helper 2 (T_H2)-type response to myelin antigens. This is desirable because T_H1-type responses to myelin proteins are pathogenic. However, T_H2-type responses are associated with allergic reactions, and about 10% of individuals taking glatiramer acetate develop allergic reactions.

An altered peptide ligand (APL) of MBP-derived peptide 83–99 was constructed by mutating the amino acids that form the main contact sites with the TCR on disease-causing T cells[43]. The administration of the

Table 1 | Therapeutics for human autoimmunity

Target/therapeutic	Status of therapeutic	Disease outcome	Disadvantages	References
Cytokines				
TNF-specific monoclonal antibody	Approved for rheumatoid arthritis, Crohn's disease, psoriatic arthritis and ankylosing spondylitis	Improvement in disability in all diseases; joint repair in rheumatoid arthritis	Increased risk of TB and other infections; slight increased risk of lymphoma	28–30, 32
Soluble TNFR fusion protein	Approved for rheumatoid arthritis, psoriasis and ankylosing spondylitis	Clinical benefit is the same as TNF-specific monoclonal antibody	Risks are the same as TNF-specific monoclonal antibody therapy	31, 32
IL-1-receptor antagonist	Approved for rheumatoid arthritis	Improves disability	Relatively low efficacy Daily injection	34, 35
IL-15-specific monoclonal antibody	Phase II trial for rheumatoid arthritis	Promising results for disability	Potential for opportunistic infection (blocks natural killer (NK) cells, CD8 memory)	36
IL-6-receptor-specific monoclonal antibody	Phase II trial for rheumatoid arthritis	Decreased disease activity	Potential for opportunistic infection	71
Recombinant type 1 interferons	Approved for relapsing/remitting multiple sclerosis	Reduction in relapse rate	Liver toxicity; influenza-virus like syndrome is common	59
Integrins				
$\alpha_4\beta_1$-integrin-specific monoclonal antibody	Approved for relapsing/remitting multiple sclerosis Phase II/III trials for rheumatoid arthritisand inflammatory bowel disease	Reduction in relapse rate; delay in progression of disability at two years; encephalopathy	Increased risk of infection Progressive multifocal encephalopathy	6, 10
Oral small-molecule inhibitors	Phase I trials in progress	Not yet known		
HMG-coenzyme A reductase				
Statins	Phase II trials for multiple sclerosis	Reduced activity on magnetic resonance scans	Hepatotoxicity, rhabdomyolysis	72
T cells				
CD3-specific monoclonal antibody	Phase II trials for type 1 diabetes	Reduced insulin usage	Increased risk of infection	14–16
CTLA4-immunoglobulin recombinant protein	Phase III trials for rheumatoid arthritis, psoriasis and multiple sclerosis	Improvement in rheumatoid arthritis		20
B cells				
CD20-specific monoclonal antibody	Phase II trials for rheumatoid arthritis, systemic lupus erythematosus (SLE) and multiple sclerosis	Improvement in rheumatoid arthritis SLE (although extensive co-medication makes interpretation problematic)	Possible increased risk of infection especially if re-treated	24
Antigen-specific T-cell responses				
Random copolymer glatiramer acetate	Approved for relapsing/remitting multiple sclerosis	Reduction in relapse rate	Allergic reactions in 10% of patients	44, 45
Altered peptide ligand to MBP peptide 83-99	Phase IIb trials for multiple sclerosis	Reduced brain lesions (at low doses)	Can exacerbate disease at high doses	46
Altered peptide ligand to HSP60 peptide	Phase II trials for type 1 diabetes	Reduced insulin usage	Allergic reactions in 10% of patients	11
Altered peptide ligand to insulin peptide	Phase II trial in progress for type 1 diabetes	Not yet known	Not yet known	
MBP-encoding tolerizing DNA vaccine	Phase I/II trial in progress for relapsing/remitting multiple sclerosis	Not yet known	Not yet known	11, 48

MBP APL ameliorated EAE in mice induced by a different myelin protein (proteolipid protein), even when the APL was administered after the initial attack of paralysis[43]. And APL administration similarly induced a shift to T_H2-cytokine production, reduced epitope spreading, and reduced the broadening of the adaptive T- and B-cell responses. In a phase II placebo-controlled human clinical trial, MBP APL (given in weekly subcutaneous doses) shifted the response of MBP-specific T cells, promoting T_H2-cytokine production (including IL-4, IL-5, IL-10 and IL-13) and downregulating T_H1-cytokine production (including IFN-γ and TNF)[44]. Lower doses of MBP APL reduced both the number and the volume of brain lesions detected with magnetic resonance imaging (MRI), but higher doses exacerbated disease in three patients and increased brain lesions[45]. A phase IIb trial is now underway using the lower dose. Three other trials of antigen-specific therapy are underway or recently completed for type 1 diabetes mellitus (T1DM), including phase II trials with glutamic acid decarboxylase, and trials with APLs of an insulin peptide or of a heat-shock protein 60 (HSP60) peptide. In the trial with the APL of HSP60, decreased exogenous insulin use was observed in diabetics, as well as a T_H2 shift[46,47].

An alternative method of targeting antigen-specific responses has recently been developed using DNA constructs that are designed to promote the tolerization of immune responses to multiple myelin components. These DNA constructs encode several myelin antigens, where immune stimulatory motifs (CpG motifs) in the DNA, which promote expression of costimulatory molecules (such as CD28), are replaced with immunosuppressive motifs (GpG motifs), leading to sub-optimal costimulation of antigen-specific T cells (Fig. 2; ref. 48). When administered to mice after the first signs of EAE, these DNA plasmids reduced the subsequent relapse rate over the next three months by more than 50%, and also reduced the spreading of autoantibody responses. A phase I trial with DNA vaccines designed to tolerize against myelin proteins is currently underway.

Oral administration of myelin antigens in multiple sclerosis, collagen in rheumatoid arthritis and insulin in T1DM (which has been shown to favour tolerization of immune responses) has been tested. Despite successfully preventing disease in animal models when antigen was fed at the time of disease induction[49,50], clinical trials attempting to treat ongoing disease have been unsuccessful[51]. A summary of therapeutics is in Table 1.

Current tools for immunotherapy

Monoclonal antibodies

The success of monoclonal antibodies was slow to arrive, but in 2004, there were two 'blockbusters' on the market (each generating over $1 billion) — infliximab (Remicade), an anti-TNF antibody, and rituximab, an anti-CD20 antibody. More are on the way; currently almost half of all drug candidates in clinical development are monoclonal antibodies. Infliximab and rituximab are derived from early monoclonal antibody technology. They are 'chimaeric' antibodies, consisting of a mouse combining site (Fv) while the rest (about 70%) is human[52]. Subsequent developments have led to 'humanized' antibodies, in which mouse-derived variable regions (or complementarity-determining regions, CDRs) are grafted into a human antibody scaffold, and 'fully human' antibodies, which contain human variable-region components selected by phage display[53]. Humanized monoclonal antibodies in the clinic include natalizumab, which blocks $\alpha_4\beta_1$ (ref. 6), and the fully human anti-TNF antibody adalimumab[30] (Humira).

Because many potential therapeutic targets are exposed in extracellular fluids (cytokines, chemokines, receptors, other cell-surface molecules and adhesion molecules), they are readily accessible to high-affinity neutralizing antibodies. Furthermore, as natural-body constituents (in contrast to the small-molecule chemicals commonly used as pharmaceuticals), antibodies intrinsically lack toxicity when manufactured, purified and handled properly. Therefore, any toxicity that does occur with monoclonal antibodies is likely to be mechanism related. Another benefit of monoclonal antibodies lies in the fact that even partially humanized antibodies (such as chimaeric antibodies of mouse Fv on a human backbone), as well as fully humanized antibodies, are relatively non-immunogenic. This is probably due to the phenomenon of 'high zone tolerance' described in the 1960s and 1970s that occurs with deaggregated human immunoglobulins[54] (whereby intravenous deaggregated gammaglobulin was tolerogenic if given in high doses) and the concomitant use of methotrexate, which has immunosuppressive as well as autoinflammatory effects[29].

Receptor fusion proteins

Receptor fusion proteins are proteins in which the binding site of a receptor is fused onto an antibody Fc region, which improves the protein's half-life and other pharmacological properties. The most successful receptor fusion protein is etanercept, a dimeric p75 TNFR–immunoglobulin G (IgG) Fc fusion protein[31] (with sales of over $1 billion). The clinical benefit of etanercept is indistinguishable from that of anti-TNF antibodies in rheumatoid arthritis[32,55], psoriasis and ankylosing spondylitis, although anti-TNF antibodies are more effective in the treatment of inflammatory bowel disease. Receptor fusion proteins are more expensive to manufacture than antibodies, and the use of natural receptors provides for less diversity than with antibodies.

Cytokines

Cytokines have some useful 'drug-like' properties, such as potency, but also some disadvantages, such as a short half-life. But the main problem with cytokines is that they have multiple effects on many cell types[25], so systemic injection of cytokines can cause undesirable effects. Thus, the efficacy in animal models of the endogenous anti-inflammatory cytokines IL-10 (ref. 56), IL-4, IL-11 and TGF-β has not translated into their use as human therapeutics, owing to their toxicity. However, the local regulated delivery of cytokines using gene therapy could make them effective as treatments. Recently, it has become possible to engineer cytokines that have enhanced half-lives and are activated only at a desired location[57,58]. Such modifications may overcome some of the inherent difficulties of cytokine therapy.

The type 1 interferons, IFN-α and IFN-β, are effective drugs and have been approved for use in viral infections, some cancers and multiple sclerosis. In multiple sclerosis, relapse rates are reduced by 30% with the administration of IFN-β (ref. 59). However, flu-like symptoms are common during therapy with IFNs, and the immunogenicity of IFNs (probably mechanism related because they upregulate antigen presentation) can limit their efficacy. IFN-β inhibits the activity of metalloproteases 2 and 9. This protease activity is required for lymphocyte homing, so when the administration of IFN-β is combined with adhesion cell blockade, lymphocyte entry into an organ may be drastically reduced[11]. In this circumstance, endogenous viruses like JC virus, which causes progressive multifocal leukoencephalopathy, may become activated with fatal consequences.

Mutated versions of cytokines can be used as decoys, inhibiting the ability of the endogenous cytokine to act on its receptor. This has been reported with TNF variants that bind to non-mutated endogenous TNF, with the resulting trimeric complex unable to activate TNFRs. In animal models, these TNF variants are effective[60].

Box 3 | Combination drug therapy in serious diseases

Because we do not know the cause of chronic autoimmune diseases, it is unlikely that any single therapy can halt or reverse all the troubling manifestations of these diseases. The way that candidate therapies are often tested — in isolation — predisposes such therapies to failure: in isolation, their effect on a highly complex multifactorial disease process may be relatively small.

Clinically, there has been marked success in the treatment of rheumatoid arthritis by combining methotrexate — an anti-proliferative folic acid inhibitor that inhibits T cells (and other cells) — with TNF inhibitory drugs[29]. This has been followed by combining methotrexate with other therapeutics, including anti-IL-6R (ref. 33) antibody and CTLA4-immunoglobulin fusion protein[20]. Methotrexate in combination with anti-TNF therapy was used in an attempt to mimic the augmented benefit of anti-CD4 and anti-TNF antibodies[79]. The lesson here, as in cancer therapeutics, is that more clinical efficacy (and less toxicity) may result from partially blocking several pathways than from complete blockade of any one pathway, which in humans is unattainable. However, certain combinations may be risky. For example, blocking TNF and IL-1 augments the risk of infection[80] and so caution is necessary to avoid diminishing the benefit:risk ratio.

It is likely that as we understand more about the rate-limiting steps or the 'tipping points' in disease processes, better combinations will be devised to maximise efficacy and to minimize side-effects, the duration of treatment and its cost.

Overcoming limitations

Although there is a lot of optimism among some circles that many new safe therapies are just around the corner, this hope belies the fact that clinical successes, where the benefits outweigh the risks, are few and far between. The failures include antibodies specific for cell-surface antigens such as CD4 and CD25, cytokines such as IL-8, fusion proteins such as the IL-1-receptor 'trap' and the TNFRp55–immunoglobulin fusion protein lenercept, and multiple antigen-specific approaches.

It is thus comforting that there are some clear successes, such as TNF blockade, that are now well established (Table 1). However, the recent withdrawal of anti-$\alpha_4\beta_1$ integrin emphasizes the complexity of reversing ongoing autoimmune disease, without provoking serious complications. Understanding the risk versus benefit relation requires more time than is usually spent in pre-clinical models, and often takes thousands of patient-years of experience to be established.

As summarized in Box 2, anti-TNF therapy of rheumatoid arthritis has marked clinical benefit, with some changes, such as reduction in tiredness, occurring within hours. This benefit occurs in most patients whose condition has not improved following other treatments, such as methotrexate. However, the degree of clinical benefit can vary considerably from patient to patient. The greatest benefit is

seen with combination therapy (Box 3). On the basis of single parameters only, such as joint swelling, all patients improve to some degree[28], but if compound parameters are monitored, such as the American College of Rheumatology (ACR) criteria (including number of swollen and tender joints, and levels of C-reactive protein), response rates vary between 50–60% in late-stage disease[29], to more than 80% in the early-stage disease[61]. In the early stage of disease, there is evidence of disease remissions, which may persist for a year or more after the withdrawal of anti-TNF therapy[61] (F. Breedveld *et al.*, unpublished observations). So early treatment may be the most beneficial and cost-effective. But there is, as yet, no evidence of a cure.

Anti-TNF therapy reduces joint pathology, even in patients showing no clear benefit according to ACR criteria. This suggests that the links between inflammation and joint damage are not fully understood. Most importantly, recent studies have documented evidence for joint repair, after TNF blockade: joint X-rays taken after one year of treatment show an improvement in joint condition compared with those taken before treatment[62,63]. It is the first example of therapy promoting endogenous repair in any reported human disease.

The most predictable problem of therapy with TNF blockade (and most other immunotherapies including anti-$\alpha_4\beta_1$-integrin antibody) is augmentation of the risk of infection. In this case, the magnitude of this risk is hard to measure because rheumatoid arthritis patients are more susceptible to infections, partly owing to the disease and partly because of other treatments. The initial incidence of tuberculosis (TB)[64] in one in every 2,000 patients treated with anti-TNF has been reduced markedly by screening and, if necessary, administration of prophylactic therapy. Other opportunistic infections are rarer, but like TB can occasionally be lethal. More common are respiratory infections. The consensus at present is that the benefit of using TNF blockade in autoimmune diseases with a bad prognosis outweighs the risks[65,66].

The risk of infection could be reduced if the duration of TNF blockade were briefer; for example, by using small-molecule chemicals of short half-life. The dilemma here, however, is to define the right therapeutic target. Attempts so far to develop inhibitors of p38 MAP kinase — a component of pro-inflammatory signalling cascades and a favourite target among pharmaceutical companies — have not succeeded, owing to toxicity. Other interesting small-molecule targets, such as IKK2 (inhibitor of NF-κB kinase 2), are also risky choices because of their presence in almost all cells. Another approach is to target the mechanism involved in the production of TNF in the joints versus that involved in the production of TNF in the immune system, but despite evidence that the mechanism differs, we do not know the molecular targets[67].

Another common side effect of TNF blockade is the induction of IgM anti-nuclear antibodies, which have been detected in many patients (15%; ref. 68), although IgG antibodies and drug-induced lupus (an antibody-mediated disease) only rarely occur (less than one in 1,000 patients). If lupus does occur, it is reversible, treatable and not nephrotoxic, so is not a major clinical problem.

Lymphomas are more frequent in patients with rheumatoid arthritis than in the normal population, especially those with severe long-standing disease. However, as severe disease is treated by anti-TNF therapy, it is not yet clear whether there is an increased risk of developing lymphomas[69] after anti-TNF therapy.

Benefit from anti-TNF blockade is not seen in all autoimmune diseases. In fact, the treatment of multiple sclerosis patients with TNF blockade, using lenercept, a TNFRp55–Fc construct which never reached the market, exacerbates the frequency of disease relapse[8], possibly by augmenting T-cell activity[70]. This discordance may be explained, in part, by the inability of the TNFR fusion constructs to penetrate the inflamed brain owing to the endothelial blood–brain barrier. Alternatively, although TNF may have a destructive role in inflammation in the brain, it may also act as a growth factor for myelin-producing cells, indicating that TNF, similar to many other cytokines, has both harmful and beneficial effects[11].

Outlook

Two decades of work defining the molecular basis of the immune response is starting to pay off in the field of autoimmunity. A whole set of 'targeted therapies' has been developed to block many steps in the immune and pro-inflammatory response. Of these, several successes have had a profound impact on patients, on our understanding of disease mechanisms and even on the pharmaceutical industry. The variety of potential therapeutic targets is enormous, but we do not know the rules that define targets of various quality, in terms of their efficacy as well as safety. Some molecules and pathways are common in many autoimmune diseases. TNF is the best-documented example. Hence, TNF blockade is an approved therapy for multiple chronic inflammatory diseases — rheumatoid arthritis, Crohn's disease, psoriatic arthritis, ankylosing spondylitis and psoriasis, with more likely to follow.

The future goal will be to improve the efficacy of immunotherapy, from the current state of partial disease control, to increased disease control, to establishing remission and eventually to cure, without increasing either the risks or costs of treatment. An important step in this progression will be achieving earlier treatment.

For now, the non-antigen-specific approaches are the ones yielding clinical benefit, with the blocking of cytokines, and possibly adhesion molecules, being the most effective. But with such non-antigen-specific approaches, the risk of opportunistic infection is problematic. In the future, non-antigen-specific approaches may be made safer by targeting them to the site of disease, for example by gene therapy. But the most obvious way to reduce opportunistic infections is to use antigen-specific therapy — a dream of immunologists for generations now. Although several attempts in the past decade have failed, we are optimistic that eventually, the molecular understanding of tolerance and immunity will progress, and the 'holy grail' of autoimmunity — long-term antigen-specific therapy — will be reached. The progress made in devising rational and effective non-antigen-specific therapy reflects the development of useful research and therapeutic tools, and provides grounds for this optimism. ■

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