REPORT

Cell therapy for autoimmune diseases: does it have a future?

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Almost all current therapeutic concepts in autoimmune diseases are based on the systemic suppression of immune functions and are not curative. The recent introduction of biologicals such as tumour necrosis factor blocking antibodies or receptors has added greater specificity to efficient management of disease by targeted suppression of rheumatic inflammation. It is evident, however, that only the elimination of the cells secreting inflammatory mediators, rather than the blockade of secreted molecules, will offer real specific therapeutic advantages in the future. Merely the elimination of such cells and also cells controlling the secreting effector cells could be curative and induce true long term remissions. We review here the state of the art and future therapeutic concepts that are based on the specific modulation of pathogenic cells that induce and sustain autoimmune inflammation. This sounds visionary, however, a variety of basic tools are at hand now. Thus, direct and specific cell therapy of rheumatic diseases will become a true alternative to conventional therapies.

On the assumption that rheumatic inflammation is driven by chronic immune reactions (fig 1), therapeutic elimination of lymphocytes inducing or sustaining such processes has been attempted in the past. Historically, systemic elimination of T helper (Th) cells with antibodies specific for CD4 or CD3 had initially seemed promising, but this did not turn out to be a realistic option due to considerable side effects.[1–3] Currently, the systemic elimination of B lymphocytes with the anti-CD20 reagent rituximab is turning out to be an extremely promising option.[4–7] Assuming that chronic rheumatic immune reactions are driven by interactions between B cells as antigen presenting cells (APCs) and Th cells as regulating cells, eliminating either of them would leave an intact population of rheumatic memory lymphocytes of the opposite type, ready to start the disease process again once the eliminated cell type has regenerated from stem cells. In line with this concept, the elimination of both cell types by complete immunoablation with cyclophosphamide in combination with antithymocyte globulin (ATG) or antilymphocyte globulin (ALG), followed by haematopoietic stem cell transplantation (HSCT) has been the only cell therapy so far with real curative potential for autoimmune diseases such as systemic lupus erythematosus (SLE) and multiple sclerosis (MS).[8] This is a risky therapy, however, in view of the severe immunodeficiency induced for quite some time and the elimination of protective and reactive immunological memory. Figure 2 illustrates how currently available antibody based therapies target various cell populations.

It is evident that at present, in particular, lymphocyte subsets are non-specifically targeted. Therefore, depending on the overall modulation of protective immunity various systemic side effects may occur and cell therapy of rheumatic diseases needs new concepts to become a true alternative to conventional therapies. The specific targeting of autoreactive cells has to be combined with a therapeutic resetting of either central or peripheral tolerance. The latter might be possible in the nearby future with the use of regulatory T cells that have been shown to have the potential to modulate autoimmune inflammation in vivo.

TARGETING ANTIGEN PRESENTING CELLS AND THEIR POTENTIAL TO ACTIVATE T CELLS

Lymphocytes and, in particular, those specific for autoantigens are not the only ''hot'' target candidate cells for specific cellular therapies in autoimmune diseases. Autoimmune reactions are set in motion with the uptake, processing, and presentation of autoantigens through APCs. The fact that at least during the primary initiation of autoimmunity professional APCs are essential implies that dendritic cells might play an important role here. Therefore it has been envisaged that blocking important costimulatory pathways such as CD80/86 could efficiently block primary activation of autoreactive T cells.[9 10] While a reagent such as soluble CTLA4-Ig is highly efficient in modulating activation of autoreactive T cells in in vitro or in vivo experimental models of autoimmunity the routine clinical use of such drugs most likely will be hampered due to their strong systemic side effects (fig 3) and the protective immune responses necessary to shield the body against pathogens would be altered drastically. Moreover, once established, the ''reactivation'' of autoreactive memory or effector T cells is less dependent on signals mediated by costimulatory molecules and thus non-professional APCs such as B cells or non-classical antigen APCs present at the site of inflammation also efficiently activate specific T cells.

However, recently, pilot short clinical trials have shown significant disease improvement in rheumatoid arthritis (RA) and that CTLA4-Ig may be surprisingly well tolerated.[11 12] Yet, data from experimental studies suggest that only acute autoimmune reactions represent targets for therapies blocking the costimulatory pathways for T cells provided by APCs. Numerous other strategies envisage the molecular ''targeting'' of APCs (and other cell types) such as modulating cell cycling, the proteasome, or the nuclear factor (NF)-κB paths.[13–17] Doubtless at present most of these approaches lack specificity for the cells they aim to target. Moreover, as mentioned above, already established autoreactive memory T and B cells may not be influenced as much as necessary.

TARGETING T CELLS IN AUTOIMMUNITY

T cells have been the target of many routine immunosuppressive treatments since a long time. Classically, various immunosuppressive drugs that interfere with intracellular signalling pathways essential for the activation and

Abbreviations: ALG, antilymphocyte globulin; APC, antigen presenting cell; ATG, antithymocyte globulin; HSCT, haematopoietic stem cell transplantation; IL, interleukin; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; Th, T helper, TGF, transforming growth factor; Treg, regulatory CD25++ Th cells

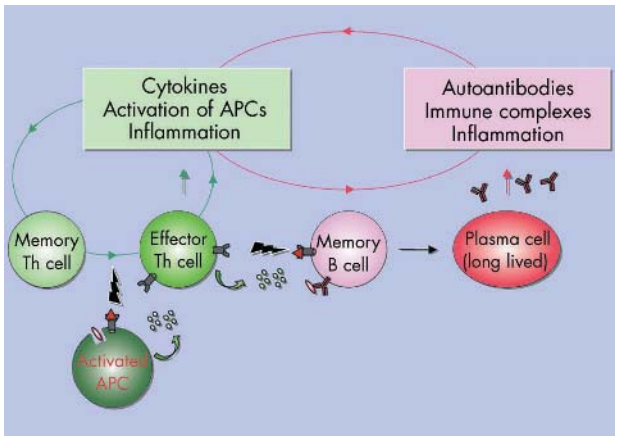

Figure 1 The chronic autoimmune reaction. Effector immunocytes as cytokine secreting effector/memory T helper (Th) cells and autoantibody secreting plasma cells are major ''players'' in chronic autoimmune reactions. In addition, activated antigen presenting cells (APCs) also play an important role in both initiating and sustaining autoimmunity. During chronic inflammation non-professional APCs as B cells also might present autoantigens.

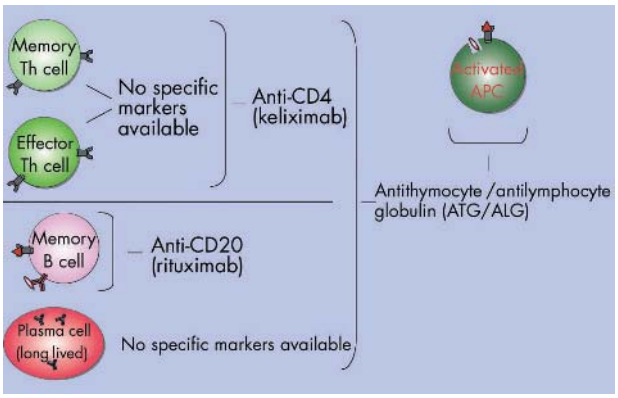

Figure 2 In vivo targeting of cells of the autoimmune reaction with antibodies. CD4+ T helper (Th) cells can be efficiently targeted by keliximab (anti-CD4). However, both protective memory and effector Th cells are affected. In addition, the targeting of naive (antigen inexperienced) Th cells (not shown) potentially decreases the overall immune competence for new pathogens, particularly since the naive T cell pools are restored slowly in older people. Rituximab (anti-CD20) is a highly efficient B cell depleting drug. However, it is not able to target long lived plasma cells since these have lost CD20 expression. No feasible antibodies for in vivo targeting of plasma cells have yet been introduced. Polyclonal antibody preparations such as ATG or ALG have been used since a long time for in vivo leucocyte depletion in haematological malignancies. Both reagents usually lead to severe and pronounced in vivo depletion of leucocytes including plasma cells and antigen presenting cells (APCs) (not shown).

proliferation of T cells have been regularly used in routine treatment for autoimmune diseases. The aim has been to interfere with Th cells that orchestrate autoimmune responses by distinctively instructing B cells and other T cells which differentiation pathways they have to enter. For instance, humoral autoimmunity is strictly in need of autoreactive Th cells since autoantibody producing B cells undergo T cell dependent affinity maturation. Moreover, autoreactive T cells and, in particular, Th cells exert important direct pathogenic functions such as secretion of various inflammatory mediators at sites of inflammation. T cell directed therapies in autoimmune diseases have therefore been envisaged and evaluated for a long time.

It has been evident, not only since naturally occurring regulatory CD25++ Th cells (Treg) gained the interest of clinical immunologists but also particularly from experimental models and from clinical experience, that depleting the pathogenic Th cells or modulating their function would be a key for inducing long term cure of autoimmunity. Most previous efforts, however, were based on the in vivo application of monoclonal antibody targeted T cells systemically (for example using anti-CD4 antibodies). Early studies with in vivo depleting anti-CD4 antibodies were mainly limited by the induction of long term Th cell deficiencies.[2 3] More recently, non-depleting anti-CD4 antibodies also have been evaluated. Here the ''coating'' efficiency of CD4+ Th cells with the drug seems to be important for suppression suggesting that anti-CD4 antibodies might be able to block Th cell interaction with APCs via a mechanism of steric hindrance.[18] Drastic systemic side effects were not described. However, after encouraging results in early open studies, randomised double blind trials still have to prove efficacy of anti-CD4 therapy or other T-cell directed antibody therapies in RA.[19 20] For all the treatments based on the systemic targeting of the whole CD4+ Th cell pool one has to consider that they might also interfere with and eliminate regulatory Th cells, not only those that arise naturally as Treg cells but also those that are generated during immune responses.

TARGETING B CELLS AND PLASMA CELLS IN AUTOIMMUNITY

B cells play a variety of pathogenic roles in human autoimmune diseases. On the one hand they may serve as potent autoantigen presenting cells and on the other hand after differentiation into plasma cells they secrete autoantibodies that through complexing antigen can promote local inflammatory reactions. Moreover, certain autoantibodies by themselves can induce direct pathogenesis, such as anti-thyroid stimulating hormone antibodies in Graves' disease, antiacetylcholine receptor antibodies in myasthenia gravis, or antineutrophil antibodies in autoimmune neutropenia.[21–23] Frequently it can be observed that certain autoantibody titres are relatively resistant despite immunosuppressive therapy with high dose steroids or even with cytotoxic drugs such as cyclophosphamide. In particular, antiphospholipid antibodies in primary antiphospholipid syndrome or anti-dsDNA in SLE can be relatively refractory to immunosuppressive therapy. The underlying mechanisms for this phenomenon have been ambiguous for a long time. Although the plasma cell source of such autoantibodies is not well characterised in humans, various experimental studies suggest that long lived autoimmune plasma cells also exist in humans and contribute to the production of pathogenic autoantibodies. However, despite the fact that plasma cells downregulate CD20 during maturation, rituximab therapy in patients with RA modulates autoantibody titres as IgG rheumatoid factor and anticitrullinated peptide antibody levels are significantly reduced while antitetanus toxic IgG is only little influenced.[6 7] Hence, the exact cellular source (long lived $CD20^{NEG}$ plasma cells or short lived CD20+ plasma cells) of individual autoantibodies in various autoimmune diseases is still a black box. It has been hypothesised that particularly in RA B cells may serve as potent local APCs at the site of inflammation.[4 5 24 25] In this case therapeutic B cell depletion with anti-CD20 removes an essential component of the autoimmune reaction that sustains the chronic inflammation leading to a reduction in autoantibody titres (fig 4).

IMMUNOLOGICAL MEMORY FOR CYTOKINES AND ANTIBODIES—THERAPEUTIC CHALLENGES

A major challenge for effective treatment of human autoimmune diseases has always been the modulation of the effector cells involved in the autoimmune reaction.

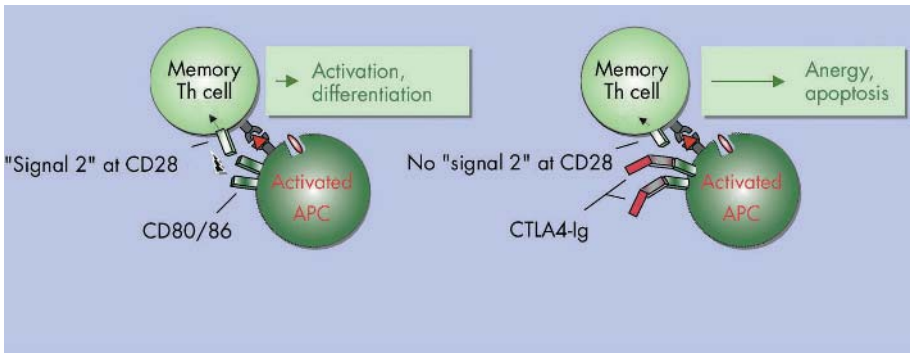

Figure 3 Targeting antigen presenting cells (APCs)—T helper (Th) cell interaction with CTLA4-Ig. During activation of T cells APCs upregulate CD80 and CD86 that can bind to CD28 on T cells and thereby provide strong costimulatory ''signal 2''. CTLA4-Ig binds with high affinity to both CD80 and CD86 molecules on the APCs and thereby blocks T cell costimulation leading to anergy or even apoptosis. However, chronically stimulated effector T cells might be less dependent on CD28 mediated costimulation. Thus CTLA4-Ig predominantly modulates primary T cell activation.

Regardless of whether humoral or cellular autoimmunity are the major players, effector cells in both arms of the immune reactions are refractory to many methods of external modulation once they have been activated by cognate interaction. Effector Th cells for instance possess a ''cytokine memory''. Once educated in a proper way to produce either inflammatory mediators (interferon γ) or Th2 cytokines (interleukin (IL)-4, IL-13) they are able to recall their initial cytokine programmes upon re-encounter with antigen. It is the stability of their epigenetic programmes that leads to limited access of gene specific transcription factors to further orchestrate expression of target effector genes. Yet, many factors in these epigenetic regulatory networks remain to be identified. The attempt to shift ''cytokine imbalances'' in autoimmune diseases using systemic or local administration of anti-inflammatory cytokines, such as IL-4, failed to show efficacy since they are not able to target and modulate the inflammatory effector arm of the Th cell compartment that drives the chronic autoimmune reaction. Moreover, effector Th cells as mentioned above are less sensitive to treatments blocking costimulatory molecules as CTLA4-Ig.

While it is still not clear whether and how effector Th cells become long lived tissue tropic memory Th cells, memory B cells unequivocally differentiate into plasma cells. Some of the effector type B cells are short lived, but an important fraction of plasma cells have been shown to be long lived.[26] Most importantly these plasma cells do not divide and are thus refractory per se to drugs interfering with proliferation of cells. Recently, the role of long lived plasma cells in autoimmune reactions has been further clarified by data from experimental models of SLE. We have shown that a considerable high fraction of plasma cells developing in the spleens of autoimmune NZB/NZBW mice are long lived.[27] More importantly we demonstrated that treatment solely with cytotoxic drugs such as cyclophosphamide was unable to target this compartment of long lived plasma cells efficiently while other splenic cell types were easily affected.[27] Treatment refractory autoantibody production has also been quite frequently described in humans. Thus as long as no specific modulation of long lived plasma cells is possible many treatments might spare the humoral effector arm of the autoimmune reaction. It is clear, however, that in case plasma cell specific treatments become feasible protective antibody titres will also be affected. Future developments might also target the homing of newly developed plasmablasts that circulate in the peripheral blood into their final survival compartments, such as bone marrow or local sites of inflammation. While the phenotype of such peripheral plasma cells has recently been assessed and various chemokines involved in plasmablast migration have been

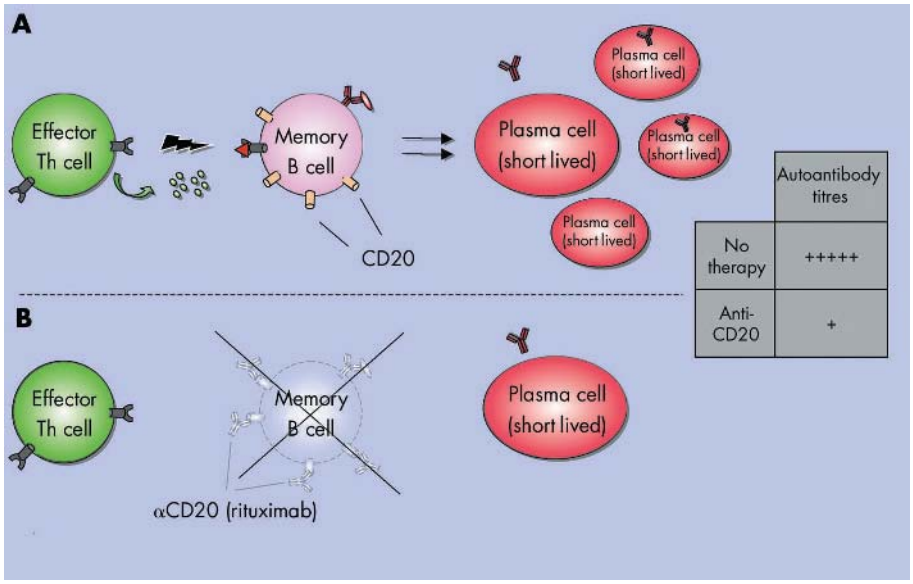

Figure 4 B cell targeting in autoimmune diseases with anti-CD20. In particular, memory B cells are eliminated during rituximab (anti-CD20) treatment. Depletion of naive B cells is compensated by swift restoration with new B cells from the bone marrow even in older people. While long lived plasma cells lacking CD20 expression cannot be targeted, the supply of pathogenic short lived plasma cells that are generated during activation of autoimmune memory B cells is reduced efficiently. Thereby autoantibody titres originating from short lived plasma cells are decreased drastically (see table) while protective antibodies remain unaltered.

described, plasma cell specific migratory or homing mechanisms have yet to be evaluated in more detail.[28]

COMPLETE IMMUNOABLATION—SUCCESSFUL BUT RISKY

The most drastic way to achieve maximal treatment efficiency in autoimmune diseases is complete immunoablation. Originally evaluated in experimentally induced autoimmunity and found by chance in patients with autoimmune diseases treated with intense immunoablation and HSCT for malignancies it has become evident that high dose treatment might offer an exclusive perspective to cure autoimmunity.[29] At first, single case reports were presented and subsequently small cohorts of autoimmune patients were enrolled in single centre studies in Chicago, Leiden, and Berlin.[30–32] Until 2004, European and international organisations collected data of several hundred cases worldwide where high dose immunosuppression combined with a subsequent HSCT was used for treatment of various autoimmune diseases. Although the numbers sound impressive, comparable clinical study designs were employed in only a minor fraction of these cases.

What have we learned from the now more than five years' experience in HSCT for autoimmune diseases? What already has been suggested in experimental animal models has been confirmed: even severe systemic autoimmunity is curable. However, in the setting of autologous HSCT, autoreactive and thereby pathogenic cells have to be targeted in vivo and also eliminated efficiently from the graft (fig 5). Today, long term remission lasting for more than six years without any treatment has been reported from different centres in particular for patients with treatment refractory SLE. Currently such results cannot be achieved with standard therapies. However, the same studies have also emphasised the major limitation of HSCT in autoimmune patients—that is, a considerable amount of treatment related complications have been reported. Therefore, even though most centres applied stringent inclusion criteria and also took extensive precautions during therapy, treatment related mortality has been reported. Obviously, at the moment HSCT is a treatment option for autoimmune diseases refractory to standard therapies.

A major finding of our studies has been that in patient responding with long term remission a drastic reactivation of thymic activity could be observed. Interestingly, such thymus driven re-establishment of a new polyclonal T cell repertoire was evident even in older patients (age >40 years at transplantation) and was a rather stable than short term phenomenon after HSCT. We assessed frequencies and absolute counts of peripheral blood CD31+ ''thymic'' naive Th cells as direct markers for thymic activity[33] in the course of the patients' immune reconstitution after HSCT. In all responding patients supranormal levels of ''thymic'' naive Th cells were detected even four years after HSCT (unpublished results). At the moment we can only hypothesise that a successful ''thymic take'' and a subsequent stable thymus dependent resetting of a new peripheral Th cell pool is highly predictive for long term remission in autoimmune patients undergoing HSCT. However, it can be foreseen that an adequate quantity of thymus derived natural Treg cells is one of the essential factors for a considerable stable restoration of peripheral tolerance for autoantigens. From numerous reports in experimental animal models it is now clear that CD25++ Tregs efficiently control the immune system potential to self-antigens, although further studies have to be undertaken to clarify the role of Treg in human autoimmune diseases.

Current HSCT study designs are not able to exclude severe side effects. Future developments for HSCT should therefore concentrate on further increasing the specificity of the immunoablation for effector T and B cells as the presently used reagent, ATG or ALG, non-specifically targets almost all leucocyte populations. Another strategy to reduce the cytotoxicity of HSCT is to isolate and preserve pathogen specific T cells from the patient prior to immunoablation to sustain protective immunity afterwards with specific adoptive T cell reinfusion. This at least should be possible in the nearby future for dominant virus pathogens as cytomegalovirus and Epstein–Barr virus. Lastly, the isolation of naive, antigen inexperienced lymphocytes (both B and T cells) prior to immunoablation might be feasible.

ADOPTIVE TRANSFER OR IN VIVO GENERATION OF REGULATORY T CELLS

As mentioned above, autoreactive Th cells are the main cell type that orchestrate pathogenic autoimmunity in many autoimmune diseases. In a healthy immune system autoreactive Th cells are also present but specialised types of natural and induced regulatory Th cells control and suppress their activities.[34 35] The underlying mechanisms represent the

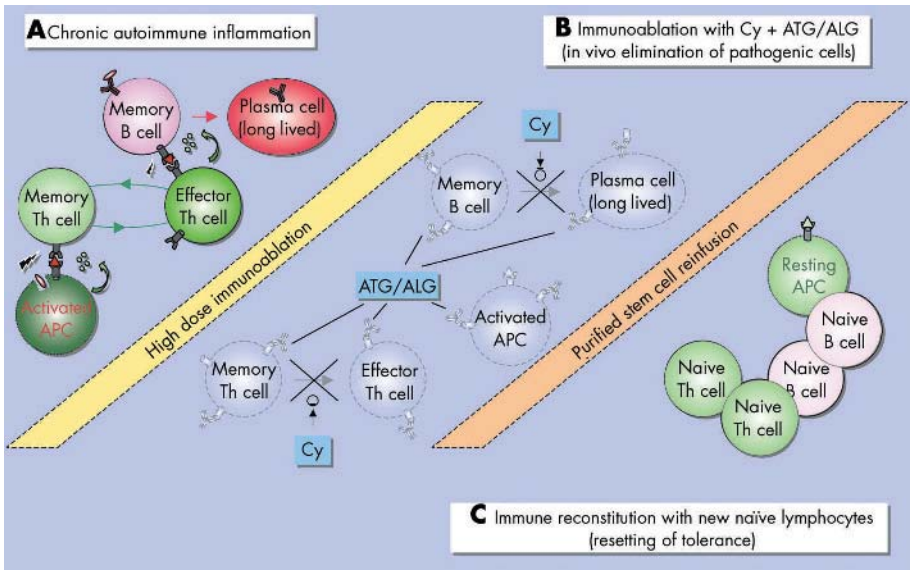

Figure 5 Haematopoietic stem cell transplantation for autoimmunity. (A) Chronic autoimmune reaction disturbs the homoeostasis of peripheral immunocyte populations. (B) During immunoablation antithymocyte globulin (ATG)/antilymphocyte globulin (ALG) efficiently eliminate resting long lived plasma cells, antigen presenting cells (APCs), or resting memory lymphocytes, and cyclophosphamide (Cy) targets the differentiation of memory B and T cells into effector immunocytes. (C) Purification of autologous stem cells prevents reinfusion with autoreactive immunocytes. Immune reconstitution with new naive B and T cells help to reset and maintain tolerance and normal immunocyte homoeostasis.

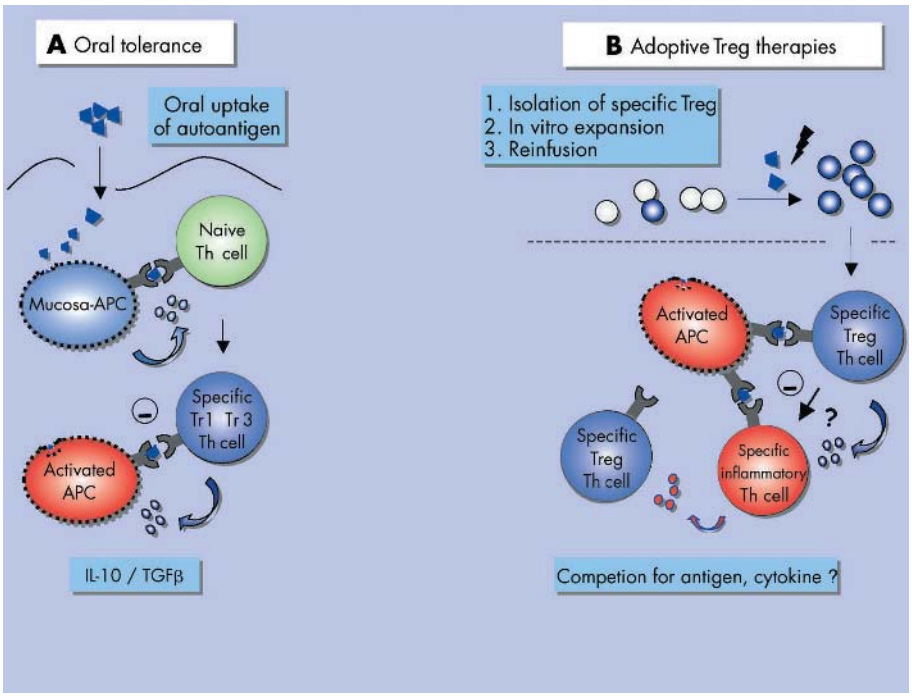

Figure 6 Control of pathogenic autoimmune reactions with regulatory T cells. (A) Uptake of target autoantigen via mucosal routes can lead to in vivo induction of regulatory T cells either producing transforming growth factor β (TGFβ) (Th 3 cells) or interleukin (IL)-10 (Tr 1 cells). Both Th 3 and Tr 1 cells target activated antigen presenting cells (APCs) presenting the autoantigen or in the end compete with pathogenic inflammatory T cells (not shown). TGFβ and IL-10 also modulate T cell functions. However, active chronic immune reactions might be refractory to modulation by oral tolerance. (B) An alternative strategy involves the isolation of specific regulatory T cells or Tregs from the peripheral blood of patients with autoimmunity. After isolation, autoantigen specific regulatory T cells can be further expanded and reinfused in high numbers back into the patient. At the site of inflammation they exert their suppressive anti-inflammatory effects after specific activation from activated APCs. Here regulatory T cells potentially compete with inflammatory T cells for antigen and growth factors but they also most likely suppress local inflammation through secretion of suppressive cytokines (for example IL-10).

most natural way to treat autoimmunity and thus their use in therapies has been envisaged for quite some time.

A different line of protection in a healthy immune system is guaranteed by tolerogenic APCs. It has been demonstrated that—for example, immature dendritic cells are able to induce the in vitro generation of regulatory Th cells characterised by IL-10 secretion (Tr 1 cells).[36] On a similar basis the immune system tolerises itself in reactions against antigens taken up via mucosal routes. Here transforming growth factor β (TGFβ) secreting Th 3 cells are induced.[37 38]

Both the natural ways of protection against unwanted autoimmune reactions would represent ideal therapeutic tools. While their basis is (auto)antigen specificity no systemic side effects have to be expected. Only the action of pathogenic (auto)antigen specific Th cells is modulated and often efficiently suppressed. While it might be not feasible to modulate an established autoimmune reaction by induction of oral tolerance specific for autoantigens (fig 6A) as has been hypothesised,[39] the adoptive transfer of ex vivo isolated or induced and in vitro expanded autoantigen specific regulatory Th cells may indeed efficiently target and suppress established chronic inflammation (fig 6B). In experimental animal models at least, such potential of Treg has been demonstrated.[40]

The greatest therapeutic advantage of Tregs has so far been the biggest limitation for their widespread use in treatment of human autoimmune diseases. Regulatory T cells or Tregs are antigen specific. However, in most human autoimmune diseases various candidate autoantigens have been characterised, but in fact often these may not represent the main targets of the inflammatory autoimmune response. This problem has been recognised and therefore a lot of effort is still being made to identify and characterise new "dominant" autoantigens. Furthermore, it has been suggested that regulatory T cells might not necessarily target the major self-antigen in an autoimmune inflammation. As soon as they are activated at the site of inflammation they exert their action non-specifically (but locally). Such bystander mechanism might work in the case of IL-10 secreting Tr 1 cells as well as for CD25++ Tregs, where the mode of suppression has yet to be characterised.

TARGETED CELL THERAPY—A REALISTIC PERSPECTIVE?

Yes, cell therapy of rheumatic diseases needs new concepts to become a true alternative to conventional therapies. Although still a vision, the combination of targeted deletion of autoreactive cells, identified according to their specificity, with the therapeutic resetting of peripheral and central tolerance mechanisms will provide a cure for rheumatic autoimmune diseases in the near future.

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