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# Immunity after organ transplantation

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## Summary

After organ transplantation an immunosuppressive regimen is required to prevent graft rejection. Immunosuppressive drugs inhibit immune function by targeting both T- and B-cell responses through blockage of cellular proliferation induced by alloantigen stimulation, and by inhibition of the cytokine production necessary for such stimulation. However, the absence of discrimination between the immune response against alloantigen from the transplanted organ and the immune response against environmental antigens renders transplanted patients strongly immunodeficient and susceptible to bacterial and viral infection. Optimising the immunosuppressive drug regimen to balance mandatory immunosuppression while preserving immunity is a difficult challenge for clinicians in charge of transplanted patients. The development and optimisation of assays to monitor the current state of an immune response is of great interest. This article reviews the mechanisms of the alloimmune response against the transplanted organ and the consequences of immunosuppression for the patient's immunity. The development and optimisation of assays for monitoring the current status of the immune response after organ transplantation is discussed, as are novel therapeutic approaches based on induction of tolerance and cellular therapy .

*Key words: organ transplantation; viral infection; immunodeficiency; HLA; tetramer*

## Introduction

The immune system protects the self against non-self aggression. Like bacteria or viruses, transplanted organs are composed of antigens against which the recipient immune system reacts, and without immunosuppression the graft is rejected. The role of immunosuppressive drugs is to inhibit the alloimmune response. The absence of discrimination between the immune response against alloantigen from the transplanted organ and the immune response against bacteria and viruses renders transplanted patients highly susceptible to infection. In haematopoietic stem cell transplantation (HSCT), good clinical experience has been gained of immune monitoring from related and unrelated donors for prediction of graft-versus-host disease (GVHD), and more recently for monitoring of immune function against infectious agents. Experience in solid organ transplantation (SOT) is less extensive, although renewed efforts are under way to detect mechanisms of tolerance and rejection. The capacity of the immune system to protect the recipient against infectious disease after organ transplantation has been less precisely analysed, and the development of assays to monitor the current state of an immune response is of great interest. These assays have the potential to identify rejection and/or to focus on the specific antiinfectious immune function without resorting to invasive tests. Such assays will also provide a more complete understanding of the mechanisms underlying the generation of tolerance, and this will open the door to new and better-targeted therapy. A reliable index of immune status could result in customisation of immunosuppressive drugs, not only in the context of rejection/tolerance, but also in the context of a strong increase in susceptibility to infections. Such an objective is highly desirable, given the morbidity and mortality associated with long-term administration of immunosuppressive therapy [1].

**Abbreviations**

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| HSCT: | haematopoietic stem cell transplantation |
| GVHD: | graft-versus-host disease |
| SOT: | solid organ transplantation |
| APC: | antigen presenting cell |
| HLA: | human leukocyte antigens |
| TLI: | total lymphoid irradiation |
| ATG: | anti-thymocyte globulin |

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## Immune response against transplanted organs

The immune response against transplanted organs arises from several genetic barriers. Blood group incompatibility is the first, and if organ transplantation across the blood barrier is performed in selected cases (e.g. kidney), ABO-compatible transplantation is the rule.

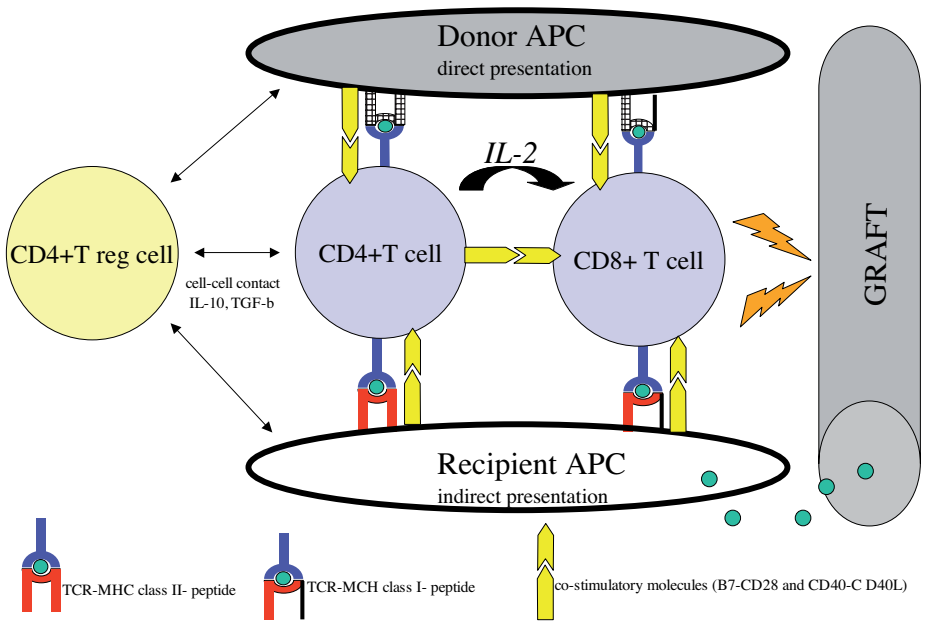
The second genetic barrier is formed by the highly polymorphic human leukocyte antigens (HLA) expressed by almost all nucleated cells. The HLA effect is most pronounced in allogeneic HSCT, where compatibility for HLA at a high resolution level has been clearly shown to be associated with better survival and a lower rate of GVHD [2]. In organ transplantation the effect of HLA matching on clinical outcome varies greatly with the organ being transplanted. In the case of liver transplantation for autoimmune disease, donor matching may actually be detrimental [3]. In renal transplantation, the benefits of HLA-A, -B, and -DR matching are still evident even with modern immunosuppression [4–6]. The better survival of transplanted kidneys with good matching is explained by the reduction of rejection episodes, leading to a reduction of the total "load" of immunosuppressive drugs, most of which have renal side effects. Good HLA matching should therefore result in less immunosuppression and better immunity for transplant patients. However, unlike HSCT, organ-transplanted patients usually have poor HLA compatibility with the donor graft tested at a low resolution level. In consequence, patients are more susceptible to HLA alloimmunisation, which is a serious problem in the context of retransplantation. Immunisation by antibodies against donor HLA may occur after blood transfusion [7], pregnancy [8] or any organ transplantation [9, 10] and sensitised patients are at high risk of hyperacute or severe acute rejection, often resulting in graft loss. In Switzerland 10% of patients on the waiting list for a first kidney transplant are immunised with anti-HLA antibodies. This number rises to 55% for those awaiting retransplantation. In these patients HLA matching is still important and is mandatory for a specific locus, to avoid humoral rejection. In this specific context of hyperimmunisation strategies have been optimised to desensitise patients before transplantation or to define acceptable mismatches [11, 12].

In addition to the genetic barrier of HLA, minor histocompatiblity antigen corresponds to a large number of polymorphic proteins expressed by every individual. Minor antigens are presented to the recipient's immune response in the context of an appropriate restriction element and eliciting of an alloimmune response. Determination of the alloimmune response due to minor antigens in the context of organ transplantation (which can be extrapolated from HLA full match patient/organ) will represent the sum of all minor antigen stimulation. Nothing can be done to prevent the alloimmune response towards minor antigens except the global immunosuppression induced by immunosuppressive drugs.

The mechanism of organ rejection involves the two pathways of alloantigen recognition (figure 1). With the "direct" pathway, intact donor HLA molecules expressed on the surface of donor cells and presenting a "normal antigen", are directly recognised by recipient T cells. This pathway can be tested by a cellular in vitro assay such as the mixed lymphocyte culture (MLC) and could be an important driver of early acute transplant rejection. A direct T-cell activation pathway is most efficiently achieved by donor bone marrow-derived antigen-presenting cells (APCs) and, most importantly, tissue dendritic cells which migrate to draining lymphoid tissue shortly after transplantation. The second pathway is referred to as the "in-

**Figure 1**

Immune response against a transplanted organ. The alloimmune response against the transplanted organ is activated by two pathways. With the direct pathway, the donor antigen presenting cell (APC) transplanted with the graft presents donor antigen (HLA molecules or minor antigen) to the $CD4^+$ and $CD8^+$ T cells which are activated in the secondary lymphoid organ. Recipient APCs migrate to the graft, process donor Ag and activate $CD4^+$ and $CD8^+$ T cells, which recognise the alloantigen presented by the self-APC in the secondary lymphoid organ. Activation is mediated by the TCR (in blue) which recognises the HLA (in red) and peptide (in green) in the presence of co-stimulation (yellow) such as B7 (APC)-CD28 (T cell) and CD 40 (APC)-CD40 ligand (T cell). The presence of IL-2 is required. By a mechanism that is still unclear (cell-cell contact or inhibition by cytokines such as TGF-β or IL-10), regulatory cells such as $CD4^+CD25^+$ are able to suppress $CD4^+$ and $CD8^+$ T-cell activation.



direct" pathway and involves the internalisation, processing, and presentation of alloantigens (donor HLA antigens or minor antigens) which are presented by recipient dendritic cells. Several groups have provided evidence that indirect allorecognition is an important driver of transplant rejection [13, 14].

More recently, the role of regulatory cells in controlling and suppressing self-antigen activation has been recognised and the same cell population has also been shown to have regulatory activities on alloantigens after transplantation. It has been demonstrated that the regulatory cells are essential for induction and maintenance of tolerance. Many types of regulatory cells have been described in a number of different systems; these include $CD25^+CD4^+$ [15], $CD8^+CD28^-$ T cells [16] and T-cell receptor $(TCR)^+$ $CD4^-CD8^-$ cells [17], as well as natural killer cells (NKT) [18]. The evidence for such cells is long-standing and comes from adoptive transfer transplant studies in which tolerance can be transferred to a naïve recipient by $CD4^+$ T cells. Although the mechanisms of this regulation remain incompletely understood, some progress has been made in defining the phenotype of the $CD4^+$ T regulatory population. These cells have the same phenotype, $CD4^+CD25^+$, as the spontaneously arising population that plays a vital role in the prevention of autoimmune disease and as activated CD4 T cells. Recently the transcription factor, foxp3, has been clearly linked to a regulatory phenotype in this $CD4^+$T-cell population in mouse and human studies [19, 20].

Organ transplantation has become an accepted form of treatment for end-stage kidney, liver, heart, pancreas and lung disease, and, to prevent immune response against the transplanted organ as described above, patients receive a combination of immunosuppressive drugs for the rest of their lives. Classical immunosuppressive regimens are based on glucorticoids, calcineurin inhibitors such as cyclosporine or tacrolimus, and more recent drugs such as mycophenolate mofetil, sirolimus or monoclonal antibody which block IL-2 receptor have contributed to the impressive one-year graft survival figures achieved by most transplant centres worldwide. Under these regimens, T-cell responses are globally impaired through blockage of cellular proliferation after antigen stimulation, as well as inhibition of the cytokine production necessary for such stimulation (see review in [21]). These drugs have little direct B-cell effect but by inhibiting T-cell response most of these regimens also have a T- dependant B-cell inhibition. Corticosteroids are potent cytokine inhibitors (interleukin-1, interleukin-2, interleukin-6, tumour necrosis factor and interferon-γ) and block antigen-induced T-cell proliferation. Calcineurin inhibitors directly inhibit interleukin-2-dependent T-cell proliferation, and blocking interleukin-4 and interleukin-5 production by T cells has an inhibitory effect on B-cell function and antibody production. Azathioprine and mycophenolate mofetil, also used as third-line agents, interfere with purine synthesis, although at different steps, blocking both T- and B-cell proliferation (see review in [21]). More recently developed, sirolimus interferes with mTOR (mammalian target of rapamycin) pathways and also inhibit T-cell activation [22]. The combination of these mechanisms leads to significant impairment of the immunological cascade following alloantigen presentation to immune cells.

## Immunomonitoring of the immune response

Monitoring lymphocyte responses to assess donor-specific immunity or tolerance uses different readouts such as proliferation, cytokine production (HTLp), or cytotoxicity (CTLp). In the context of bone marrow transplantation such tests are used in several centres for the prediction of GVHD and also for donor selection. In the beginning this need was driven by the lack of high-resolution tissue typing techniques. Estimation of host-reactive CTLp in the peripheral blood of donors has been shown to be predictive of acute GVHD and survival in a series of studies [23, 24]. Host reactive IL-2 HTLp frequencies have been shown to correlate with outcome in identical siblings and unrelated donors [25, 26]. In all these studies, where donor and recipient share the majority of HLA molecules, the usefulness of in vitro assays remains controversial but such assays may help to define permissible mismatches in the absence of a completely matched donor.

In solid organ transplants the data are less abundant, and conflicting data have been reported on the ability of CTLp measurements to predict rejection [27, 28]. Reduction of immunosuppression should be a major objective for every transplanted patient in order to reduce the drugs' side effects and restore immunity against common infectious agents, and CTLp measurements have been used to assist in deciding to reduce immunosuppression in selected cases [29]. Albeit labour-intensive and complex, in vitro assays are still a valuable tool in monitoring donor-specific responses, particularly in the era of computerised calculations. Their specificity and relationship to clinical outcome have not been surpassed by any other assay to date. Their ability to unmask regulatory cell effects and the range of measurable readouts will ensure their continued usefulness.

To analyse the immune function against infection, tetramer technology has, by showing the presence and function of $CD4^+$ and $CD8^+$ T cells against specific viruses, revolutionised the visuali-

sation and quantitation of antigen-specific T-cell response against infectious agents. HLA-peptide tetrameric complexes allow direct ex vivo visualisation of antigen-specific $CD8^+$ or $CD4^+$ T cells by flow cytometry [30]. Quantitation, phenotypic analysis and isolation of tetramer-binding specific T cells have proved clinically useful in the monitoring of immunity to infectious diseases caused by different viruses, such as HIV [31], hepatitis-B virus or CMV [32] and in tumour immunology. However, tetramer technology is HLA-dependent, and this is a limitation since it is only routinely available for limited HLA antigens and consequently for a limited number of patients, nor does it provide any indication of the functionality of the specific T cells. Other approaches are available, based on antigen-specific T cells analysed and isolated using the IFN-γ secretion assay. Whole blood, PBMC or other leukocytes are stimulated for a short period of time with specific peptide, protein or other viral antigen preparations. The secreted IFN-γ binds to the IFN-γ receiving reagent on the positive, secreting cells. More recently, new technology has directly combined specific detection with tetramer analysis and intercellular cytokine staining, providing both function and antigen specificity.

These new approaches to detection of T or NK cells against specific infectious agents can be combined with the technology available to measure cytokine secretion (by ELISA or Elispot) [33] or to quantify cell division after stimulation with CFSE (carboxyfluorescein succinimidyl ester labelling) [34]. The phenotype of these cells can also be analysed at the mRNA level. Several technologies are available which detect a pattern of hundreds or thousands of genes by microarray [35] and/or to quantify gene expression by real time polymerase chain reaction.

## Therapeutic strategy for preservation of immunity after organ transplantation

The immunosuppressive regimens used after organ transplantation are efficient but, as a result of their non-specific mechanism of action, they fail to prevent chronic graft rejection, life-threatening infections and malignancy. The "perfect" immunosuppressive regimen would specifically inhibit anti-graft alloimmunity but preserve immunity against bacteria and viruses. This objective is close to the definition of tolerance, which was originally defined as long-term allograft survival in the absence of immunosuppressive drugs. The donor-specific unresponsiveness observed in the tolerant state goes together with the persistence of third party response in functional assays, meaning that the immune response against any foreign antigen (except those expressed by the graft) is preserved. The immunological mechanisms of tolerance induction towards an allograft are basically the same as those which maintain tolerance to self-antigen: central or peripheral deletion, anergy, regulation/suppression and ignorance (figure 2). On the basis of these mechanisms, several therapeutic strategies have been tested to induce tolerance in organ transplantation.

(a) The first is induction of peripheral tolerance by depletion of lymphocytes. Because graft rejection is mainly mediated by $CD4^+$ and $CD8^+$T cells, lymphocyte depletion at the time of organ transplantation has been advocated by some as a strategy for reducing the rate of rejection [36, 37]. This strategy began many years ago with total lymphoid irradiation (TLI), and was then combined in animal studies with anti-CD3 or anti-CD4. Non-human primate studies have also suggested that T-cell depletion at the time of transplantation may substantially promote long-term unresponsiveness [36]. In humans, TLI was used in combination with anti-thymocytes globulin (ATG) in a small number of patients, and a few became tolerant. The more common experience of T-cell depletion

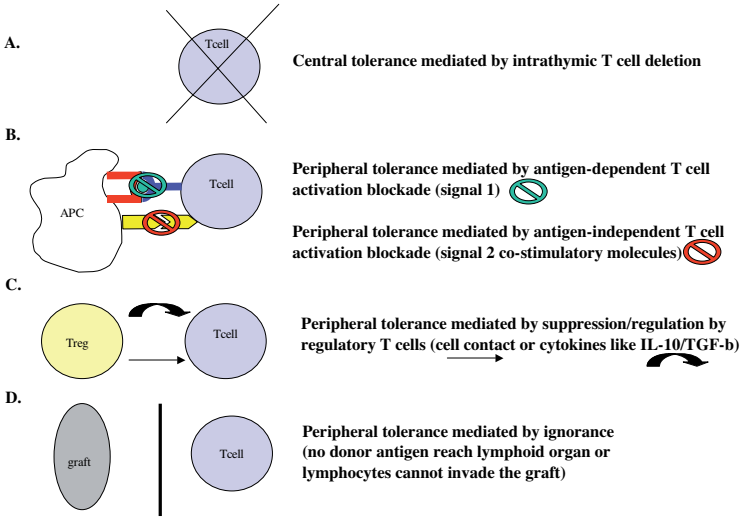



**Figure 2**

Mechanism of tolerance induction in organ transplantation. **(A)** Central deletion can be achieved by direct injection of donor cells into the thymus but in clinical practice colonisation of the thymus by donor haematopoietic cells ensures a continuous supply of donor antigen in the thymus leading to negative selection of the immature T cell. **(B)** Peripheral tolerance can be achieved by the depletion of T lymphocytes with monoclonal antibody to remove alloreactive T cells without specificity. Blocking the co-stimulatory molecules prevents T cells from activation, leading to anergy. **(C)** Regulatory T cells inhibit T-cell activation by cell-cell contact and/or secretion of anti-inflammatory cytokines such as TGF-β or IL-10. Regulatory T cells could also maintain dendritic cells in an immature and tolerogenic state [55]. **(D)** Ignorance is achieved in very specific conditions such as non-vascularised organ transplantation (i.e corneal allograft).

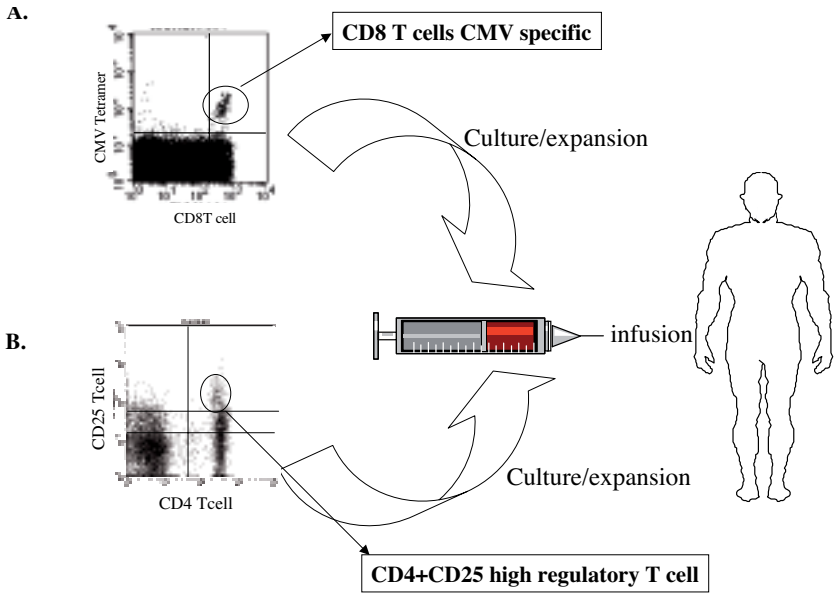



**Figure 3**

**Cellular therapy and organ transplantation**. (**A**). CD8 specific T cells can be detected in CMV positive patients by a tetramer. CD8 specific T cells can be selected, cultured ex vivo, amplified and reinfused into immunosuppressed patients who have escaped antiviral therapy. (**B**): $CD4^+CD25$ high T cells have regulatory properties. $CD4^+CD25$ high T cells can be selected, cultured ex vivo, amplified and reinfused into the patient to induce tolerance or to treat rejection.

in kidney transplantation is with Campath-1H (alemtuzumab), a monoclonal antibody directed against the CD52 protein expressed at the surface of T cells. Approved for the treatment of certain types of leukaemia, Campath has now been used in more than 100 kidney transplanted patients in combination with other immunosuppressive drugs [38, 39]. Lymphocyte depletion with Campath-1H appears to be effective in preventing rejection and so far has been quite safe from the infection/malignancy standpoint. However, cellular and also strong humoral rejection episodes were observed in several patients and it was important to realise that intensive T-cell depletion did not induce tolerance. Other promising depleting strategies using anti-CD3 coupled with an immunotoxin are under investigation.

(b) To induce peripheral anergy, costimulation blockade is another strategy for promotion of graft acceptance in transplantation, and one which has the advantage of being associated with very few toxicities. A recent study shows that belatacept (CTLA4-Ig), an investigational selective costimulation blocker of the B7-CD28 pathway, did not appear to be inferior to cyclosporine as a means of preventing acute rejection after renal transplantation. Belatacept was used with other immunosupressive drugs in this study [40]. Promising initial studies with a monoclonal antibody which blockaded the CD40-CD40L pathway (anti-CD154) were performed in non-human primates. Graft survival was greatly prolonged [41, 42] but true tolerance was not achieved. In humans, anti-CD154 has begun testing in clinical trials but this monoclonal antibody was associated with an increased incidence of thrombotic side effects [43].

(c) Other strategies based on coinfusion of haematopoietic cells and organ transplantation have been proposed for induction of tolerance. Reports on donor lymphocyte infusion (DST) and infusion of cadaveric bone marrow have been published and in some studies a tendency to better long-term survival of the graft is observed, with a significant reduction of immunosuppressive drugs in some patients [44]. However, no effect was demonstrated in other reports [45].

Recently, in kidney living donation, new approaches based on infusion of haematopoietic stem cells to achieve immunological tolerance have been optimised in clinical trials by two groups. The idea of haematopoietic stem cell infusion is based on the hypothesis that donor-derived haematopoietic cells can reach the recipient thymus and promote negative selection of newly generated donor-reactive T cells leading to central tolerance. The animal models developed to set up this strategy have demonstrated that mixed allogeneic chimaerism may induce a reliable and robust form of tolerance. In the patient, bone marrow or peripheral stem cell infusion could be acceptable only if low toxicity regimens for achieving mixed chimaerism are developed. A group in Stanford used a conditioning regimen consisting of total lymphoid irradiation and ATG followed by transplantation of G-CSF-mobilised HLA-mismatched $CD34^+$ cells and a kidney from the same donor followed by post-transplant immunosuppression [46]. Two out of four patients were off all immunosuppressive drugs after 12 months. They subsequently developed acute rejection episodes and immunosuppressive therapy had to be resumed [46]. Thus, tolerance was not achieved. The Massachusetts General Hospital has optimised a protocol that includes combined kidney transplantation and non-myeloablative HCT from HLA-identical related donors to multiple myeloma patients in renal failure, using a regimen involving peritransplant ATG, cyclophosphamide, thymic irradiation and a short post-transplantation course of cyclosporine [47, 48]. The results for two patients have been published and are highly encouraging, showing that tolerance has been achieved at 3.5 and 5.5 years after transplantation. A second protocol involving HLA-haploidentical transplanta-

tion is also under investigation at the Massachusetts General Hospital.

(d) Trials to identify regulatory T cells (Treg) in long-term kidney transplant recipients have already started [49, 50]. Tracking the expansion or depletion of Treg in transplant patients may therefore enable immunosuppression protocols to be reevaluated in the near future. Ex vivo strategies for generation and/or clonal expansion of the regulatory T cells from transplant recipients is another exciting approach which highlights the future potential for cellular therapeutic agents (figure 3a). In animal models, treating GVHD with expanded regulatory cells seems a promising approach [51, 52], but careful study of Treg generated by these strategies in in vivo models, together with clinical trials, is essential to ensure safe and smooth induction of tolerance to donor alloantigens in the future.

In the emerging field of cellular therapy the preservation of antiviral immunity by immunotherapy with large scale culture and amplification of virus specific $CD8^+$ T-cells (figure 3) has shown promising results [53], but this approach will be confined to a small number of patients who have escaped antiviral therapy without cellular immune protection and have a potentially life-threatening viral infection (figure 3b).

Finally, with a view to preserving immunity one should bear in mind the simpler approaches which can be applied to a large cohort of transplanted patients in order to minimise the amount of immunosuppressive drugs after organ transplantation. Due to the plethora of evidence implicating steroids in complications following organ transplantation, many trials have been performed with the goal of either withdrawing steroids after a long period of use or after only short-term use, or avoiding them altogether in de novo transplants. For the same reason, and also in view of their financial cost, clinical trials designed to withdraw calcineurin inhibitors have been published and have been associated with an acceptable incidence of rejection following withdrawal [54].

## Conclusions

Preserving immunity by minimising immunosuppression or inducing tolerance is one of the major goals of the transplant immunologist. Several strategies are exciting, but further work is necessary to find the best protocol to induce tolerance. Studies in human renal transplantation have illustrated the difficulties in translating non-human primate model success into the clinical arena. Redundancy of the immune system, species differences that make tolerance more difficult to achieve in higher species, and species-specific complications have contributed to the difficulties in introducing such new approaches in the clinic. Defining the ideal strategy(ies) for inducing tolerance and/or minimising the role of immunosuppressive drugs, and development of assay(s) to measure tolerance and immunity, are among the most important challenges in organ transplantation over the next few years.

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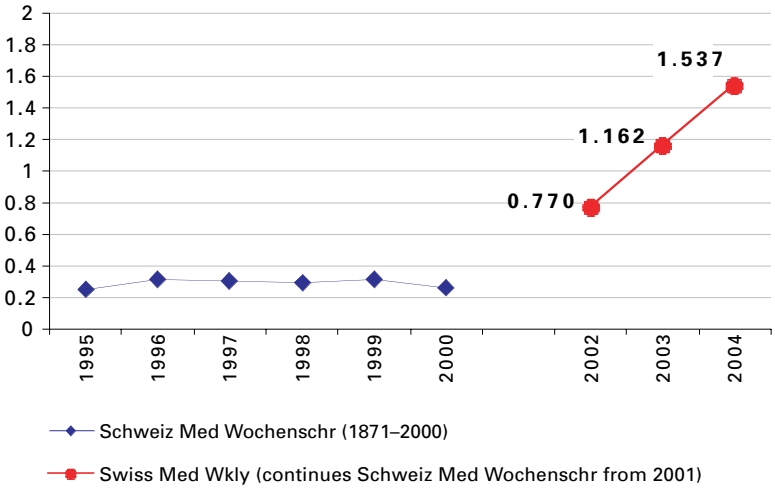


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