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NEPHROLOGY FORUM

Relevant targets for therapy with monoclonal antibodies in allograft transplantation

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Case presentation

A 45-year-old woman had been dialyzed for 3 years for end-stage renal disease secondary to polycystic kidney disease. She had received two transfusions and had developed antibodies reacting with 20% of the local screening panel. The patient was offered renal transplantation and agreed to enroll in a randomized study to evaluate the efficacy of a monoclonal antibody (mAb), directed against an adhesion/co-stimulatory molecule, CDlla, in improving graft survival. In this study, the antibody, which had yielded encouraging results in a recent pilot study, was compared (in an induction protocol) with anti-thymocyte polyclonal globulins. The protocol called for the monoclonal antibody to be administered at 20 mg/day for 10 days, along with 1 mg/kg of steroids (tapered every week, until complete withdrawal at day 45) and 2.5 mg/kg of azathioprine; cyclosporine A (CsA) was begun at the end of the mAb administration. At day 2 post transplantation, the patient excreted 2 liters of urine; her serum creatinine level decreased progressively to levels below 150 μM (1.6 mg/dl) at day 9. No adverse effect of the mAb was noted. Circulating trough levels reached 7 μg/ml at day 6 and peaked at 11 μg/ml at day 11. No changes in white blood cell counts were noted after an initial decline of 20% in lymphocyte count. As early as day 3, LFA-1 site occupancy of the patient's lymphocytes by the antibody was saturated. In addition, patient-activated T-cells had lost their capacity to bind a B-cell line bearing ICAM-1, the ligand of LFA-1. An almost total modulation of the patient's CD11a molecule at the lymphocyte surface membrane was found at the end of the treatment. All these biologic effects disappeared by day 30.

The patient did not have any rejection episodes during the first 3 months after transplantation; her serum creatinine concentration was 130 μM (1.4 mg/dl) at that time.

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Discussion

PROFESSOR JEAN-PAUL SOULILLOU (*Director, Institut de Transplantation et de Recherche en Transplantation, INSERM, Nantes, France*): Immunosuppressive drugs work by interacting with intracellular molecules involved in many biologic functions. These drugs include steroids, anti-nucleic acids (azathioprine, Brequinar, ester of mycophenolic acid, desoxyspergalin), and anti-transcription factors (cyclosporine, FK506, rapamycin). Milstein and Kohler's pioneering work on cell hybridoma initiated the era of monoclonal antibody therapy [see Ref. 1]. By recognizing a single epitope on molecules expressed at the membrane of immunocompetent cells, this immuno-intervention theoretically is restricted to a subset of immunocompetent cells and possibly to a specific biologic function and thus has considerably increased the specificity of the immunosuppression.

Anti-lymphocyte reagents used in allograft transplantation were first confined to rabbit anti-lymphocyte polyclonal gamma globulins (ALG) [2], but now a growing family of potentially useful monoclonal antibodies (mAbs) is available that recognizes a variety of targets not solely on lymphocytes. The diversity of this group of antibodies depends only on our knowledge of molecular interactions at the immunocompetent cell surface. Although well recognized as being effective in preventing and treating rejection, ALG is associated with several disadvantages. These include variability among batches, broad reactivity with antigens unrelated to lymphocytes, and the occurrence of serum sickness. Therapy with carefully selected monoclonal antibodies should circumvent these problems, as mAbs have a single defined specificity, a unique target, and are used at low dosage, thus virtually eliminating serum sickness. In addition, their effectiveness usually can be followed through specific monitoring, as exemplified in the patient presented and in Figure 1. The use of bioreagents in rejection prophylaxis protocols is of primary importance in preventing the recipient immune response during the first weeks after transplantation. In renal transplantation, this approach allows the initiation of cyclosporine A to be delayed until stable graft function is obtained, with control of the immune response by the mAb at the time of maximal risk of rejection. This approach usually is associated with better functional results, increased patient comfort, and shorter hospitalization time [3]. However, a higher incidence of viral diseases and lymphoma also has been encountered [4]. But a monoclonal antibody that has proved effective in preventing graft rejection when administered as an induction treatment is not necessarily successful in treating acute ongoing

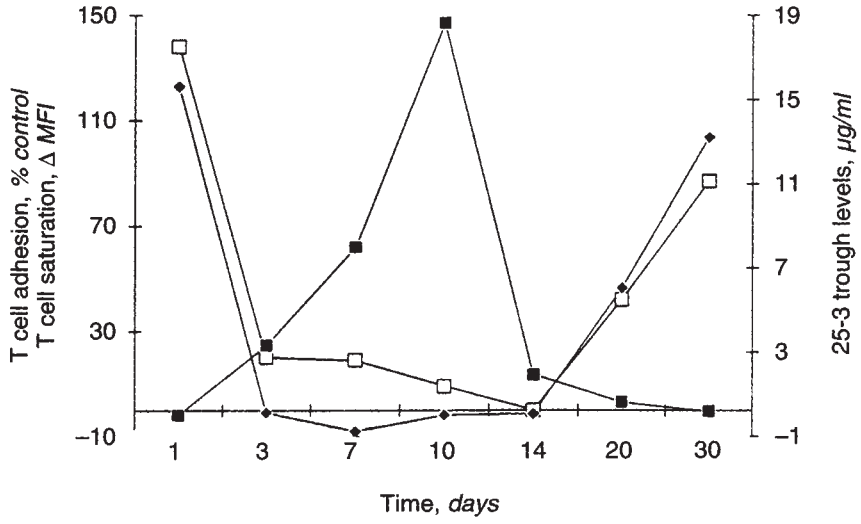

Fig. 1. *Example of the monitoring of the effect of an anti-CD11a in a human.* Besides basic pharmacodynamic information obtained from the measurement of the monoclonal antibody trough levels (■), other information specifically related to the specificity of the mAb and the function of the molecules recognized can be studied, for instance, inhibition of T-B cell adhesion (□) and CD11a site occupancy (◆). T-cell saturation by the mAb is expressed as the difference in mean fluorescence intensity (△ MFI) when patient cells are labeled in vitro with FITC-GAM with or without previous incubation with an excess of 25.3 mAb.

rejection, as has been demonstrated for an anti-IL2R and an anti-CD11a mAb [5, 6].

To date, only Orthoclone OKT3 (OKT3) has been used on a large scale in the treatment (and later, prevention) of rejection in human allograft recipients [7]. Many other monoclonal antibodies directed at several different surface membrane molecules involved in immune recognition are being actively explored in experimental animals, and some of them are under study in pilot or randomized clinical trials. In this Forum, I will focus on the mAbs already used in the treatment of human allograft recipients. I also will briefly discuss the new possibilities offered by some mAbs still under study in animal models, as well as the new therapeutic tools derived from monoclonal antibodies that have emerged from molecular biologic techniques, such as chimeric or humanized mAbs, or fusion molecules that comprise a part of the immunoglobulin (Ig) structure.

Rationale for the choice of potential targets

Experimental data in animals indicate that therapeutic options can vary according to the type of organ transplanted, the class I- or class-II compatibilities, and the magnitude of immunosuppression required by the clinical situation (for example, preimmunization). Therefore, different reagents are required to exert various effects on the recipient's immune response and to act at different steps.

Murine IgG monoclonal antibodies have poor and sometimes undemonstrated in-vitro effector functions such as complement-dependent killing, antibody-dependent cytotoxicity, and opsonization. Thus, antibodies directed at "functional" surface-membrane molecules have been preferred to those recognizing "structural" targets, for example, anti CD7 [8–11], T12 or CD6 [12], or CBL1 antibodies [13], not primarily involved in immune recognition or effector functions of immunocompetent cells. Furthermore, some antibodies, highly specific for a function, by interacting with a minority of immunocompetent cells, might gain in specificity and produce fewer side effects by delivering inhibitory signals only, without destroying their target (Table 1). The interpretation of these data has been complicated by the observation that some mAbs could induce tolerance rather than classic immunosuppression. Although this concept is extremely attractive, the salutary

Table 1. Specific versus nonspecific effects of monoclonal antibodies

Ligand/receptor inhibition
IL2/IL2R
ICAM/LFA-1
Membrane modulation
CD3/CD4/CD7
LFA-1
Complement killing (isotype-related)
ADCC and promiscuous killing
CTL
Monocytes/macrophages
NK, CD16+ cells
Target opsonization
Apoptosis

state of tolerance has been achieved only in rodent allografts. In this model, the tolerance state, as defined by a long-term acceptance of donor tissues with rejection of third-party tissues can, however, be produced by different immune manipulations, including pregraft donor blood transfusions [14], administration of CsA [15], anti-ICAM/LFA-l [16], anti-CD4 mAbs [17], and may result from efficient early immunosuppresion rather than from a specific effect of the mAbs used.

Our ability to employ specific mAbs in vivo in animal models and in some clinical trials is governed by our current knowledge in the field of immune recognition and effector functions, especially our understanding of the T-cell receptor complex, the growth factor receptor involved in the clonal expansion of committed cells, and adhesion and "second signal" molecules. These various target families respond to different stimuli and have various molecular interaction requirements; in some instances, the mAb acts directly by modulating a ligand at the cell membrane (for example, anti-CD3), whereas others act mostly as competitors for soluble (for example, anti-IL2R) or membranous ligands (for example, anti-LFA-1, anti-ICAM-1). In the last examples, the epitope recognized is of paramount importance in achieving a high degree of functional inhibition. The search for important targets is not over and we are still in a phase of exploring potentially interesting reagents (Fig. 2).

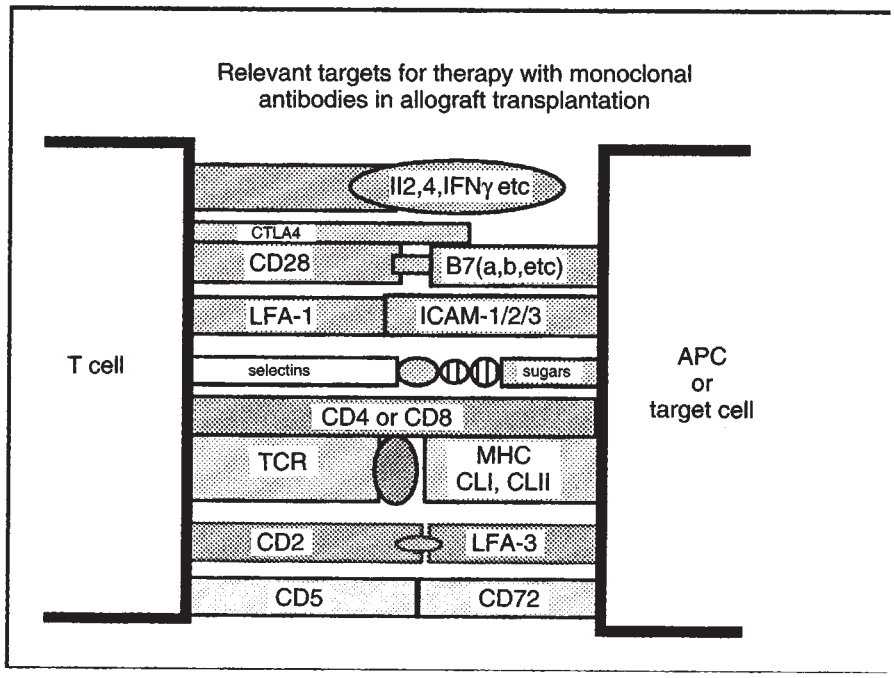

Fig. 2. *Examples of molecules used as targets of monoclonal antibodies in immunosuppression* in vivo, *or those of potential interest.* From top to bottom: receptors for T-cell growth factors (IL2, IL4, for instance) or for key factors in inflammatory responses (IFNγ); "second signal" molecules such as CD28 or CTLA-4 and their counter-receptors of the B7 family; adhesion molecules such as β2 integrin (LFA-1), selectins (such as L-selectin on lymphocyte or E-selectin on graft vessels) and beneath the T-cell receptor; the CD2/LFA-3 couple. The molecules (CD2/CD72) indicated at the bottom of the figure are given as examples of targets that could be used to specifically inhibit T-helper/B-cell cooperation.

Anti-T-cell receptor complex

Anti-CD3 monoclonal antibodies. Orthoclone OKT3 [7] is a mouse IgG2a directed against the 20 kD glycoprotein chain of the CD3 complex (epsilon chain), which flanks the T-cell receptor [see 18 for review]. First used in renal transplant patients by Cosimi et al in 1981 [19] and marketed in 1986, OKT3 is now widely recognized as a powerful immunosuppressive agent that reverses acute rejection [20] and is as effective as ALG in protocols designed to achieve prophylaxis [21]. In these studies [19, 20], OKT3 was given intravenously at a daily dose of 5 mg for 10 to 14 days, but lower doses (such as 22 mg over 10 days) have yielded good results in induction protocols of renal transplantation [22]. Whereas high doses of steroids reversed 75% of acute renal rejection episodes [7], OKT3 reversed 94% of acute rejection episodes; OKT3 also was effective in rejection episodes resistant to steroids or ALG [23]. The effect of OKT3 on renal function is somewhat delayed, however, occurring within 20 days after the onset of the treatment, and even longer in the case of steroid- or ALG-resistant rejection episodes [22]. Recurrent rejection episodes occur frequently (66% of patients) when OKT3 is administered without CsA [7] but only in 33% of patients given CsA [24].

In protocols designed for prophylaxis, cardiac transplant recipients treated with OKT3 experienced fewer acute rejection episodes during the first 3 months post transplantation than did those treated with ALG (1.5 ± 0.2 versus 2.2 ± 0.2 rejection episodes per patient) and required less chronic maintenance immunosuppression [25]. A randomized prospective trial in renal transplantation showed that OKT3 and ALG produced similar results [26].

The OKT3 monoclonal antibodies interact only with T-lymphocytes and therefore are more specific than ALGs, which also bind to monocytic, natural killer (NK) and B cells. In vitro, OKT3 inhibits both the generation of functional effector T-cells and the activity of mature cytotoxic effector lymphocytes [27]. Peripheral blood lymphocyte counts drop dramatically, but transiently, after OKT3 administration, and reappearing T-lymphocytes fail to express CD3 or T-cell receptors, whereas the other T-cell surface determinants are normally expressed [28]. This modulation is rapidly reversible after cessation of OKT3 or when patients are immunized against the mAb. Accordingly, monitoring of OKT3 treatment can be based on measurement of the magnitude of the decrease of the ratio of $CD3^+/CD2^+$ circulating cells and, to a lesser extent, on the measurement of the OKT3 plasma levels, even though levels of ≃1 μg/ml correspond to a concentration that blocks T-cell functions in vitro. But OKT3 administration at the usual doses does not always completely eliminate $CD3^+$ cells in the graft [29], and changes in the local T-lymphocyte populations, with a shift toward an increase in $CD8^+$ and $2H4^+$ cells, have been reported [30].

The major problem associated with OKT3 is that severe adverse side effects occur in a majority of patients, starting 45 to 60 minutes after the first two or three OKT3 injections and lasting for several hours. These sequelae can include OKT3-mediated nephrotoxicity, with an average increase of 31% in serum creatinine levels before improvement [31]. Pulmonary edema can be prevented if any existing fluid overload is corrected before treatment [7]. These side effects are related to the massive, although transient, polyclonal release of cytokines such as TNFα, IFNγ, IL2, IL3, and IL6 [32] that occurs before CD3 modulation. Three main, although not mutually exclusive, mechanisms could explain this release of cytokines: (*1*) OKT3 causes opsonization, trapping, and lympholysis of OKT3-coated cells by macrophages with a subsequent release of cytokines; however, other anti-T-cell mAbs (anti-LFA-1, -CD2, -CD4) do not provoke this phenomenon; (*2*) OKT3 induces T-cell activation in vitro and in vivo; and (*3*) The released lymphokines can, in turn, activate macrophages or, alternatively, OKT3 can bridge T-lymphocytes and macrophages/monocytes, which also results in their activation [33]. The side

effects are reduced by the administration of a high dose of steroids (a bolus of 0.5 to 1.0 g methylprednisolone intravenously one hour before injection [34]); this maneuver inhibits production of cytokines but can contribute to over-immunosuppression and confound assessment of the effect of the monoclonal antibody. The finding that anti-TNFα mAbs also decrease OKT3's side effects in mice and in humans confirms the major involvement of TNFα in this syndrome [35]. Beside their role in OKT3-induced first-dose reactions, cytokines released after OKT3 injection also might be involved in the immunosuppressive properties of the antibody. Indeed, OKT3 in humans and a similar anti-CD3 antibody in mice induced IL-10, a cytokine with potent immunosuppressive and antiinflammatory properties [36, 37].

Although no serum sickness is observed following the use of OKT3, the xenosensitization can totally abrogate the mAb's effectiveness. Recipient anti-OKT3 (IgM and IgG) of both anti-isotype and neutralizing anti-idiotype specificities are usually produced [38]. The incidence of anti-OKT3 antibodies decreases with the use of associated immunosuppressive treatment: 70% to 100% immunization was reported when OKT3 was used alone [7], compared with 25% when OKT3 was given in association with low doses of steroids and azathioprine [39], and with 15% when steroids and azathioprine were combined with CsA, the latter given at 50% of its usual maintenance dose [24].

The incidence of infectious disease does not differ between OKT3 and ALG therapy [26], but this incidence did increase significantly when OKT3 was compared with conventional immunosuppressive drug treatment [40]. Finally, two reports show that prolonged use (or re-use) of OKT3 must be avoided because it has been associated with a dramatic increase in lymphoproliferative disorders in cardiac transplantation (36% in patients who received more than 75 mg versus 6% of patients who received less than 75 mg) [41, 42].

Several other monoclonal antibodies directed against CD3 or against T-cell receptor monomorphic epitopes have been tested in human renal transplantation. While these newer anti-CD3 monoclonal antibodies did not significantly reduce cytokine release [43], progress may come from using mAbs against the monomorphic determinant of $\alpha\beta$ chains of the T-cell receptor [44], which can reverse acute rejection episodes and yield fewer and less severe side effects than expected. The use of this anti-T-cell receptor monoclonal antibody is associated with an absence of in-vitro mitogenic effect; the antibody does modulate the CD3-T-cell receptor complex at the T-lymphocyte surface. Another anti-$\alpha\beta$ chain T-cell receptor mAb (BMA 031, a murine IgG2b) was also studied as prophylaxis but was ineffective at the tested dose [45]. Another approach uses the F(ab')2 fragments, which neither cause T-cell activation nor increase animal morbidity or mortality, but which prolong the survival of skin allografts [46]. Molecular biologic techniques also can produce variable immunoglobulin fractions (scFv) [47]. Although OKT3 F(ab')2-digested fragments are markedly less potent T-cell activators in vitro than is whole mAb, no one has demonstrated in humans that monoclonal antibody fragments are effective.

Antibodies against adhesion molecules

A rapidly growing list of molecule families playing a role in cell/cell adhesion—including immunocompetent cell interrelationships and immunocompetent cells/allogeneic target interactions—has been described [see 48–50 for reviews]. Although usually ubiquitously distributed, some adhesion molecules are more restricted to bone marrow white cell lineage (such as LFA-1) or, even more specifically, to a single cell interaction, such as ELAM [51]. These molecules therefore are involved in a variety of basic biologic events, including embryo development, tissue repair, and immune function. Elegant studies have shown that dynamic leukocyte/endothelial cell interactions are regulated by several specialized receptor/ligand couples that operate in successive steps; these steps encompass a number of processes from induction of leukocyte rolling on endothelial cells to their immobilization, processes that allow immune effectors to act on endothelial cells or to leave the vascular compartment through the endothelial layer [51]. Agents that can block these receptor/ligand interactions also might interfere with immune recognition and effector functions. Furthermore, indirect experimental evidence suggests that adhesion molecules also could play a role in nonspecific white-blood-cell-related lesions of endothelial cells during the revascularization syndrome of transplants [52]. Experimentally, antibodies directed against adhesion molecules such as anti-CD2 [53], anti- LFA-1, and anti-ICAM-1 [16] have delayed or reversed graft rejection. Furthermore, an anti-CD11a associated with an anti-ICAM-1 can induce tolerance in rodents [16].

Among the adhesion molecules, those of the β_2 integrin family, including LFA-1, are involved in the late adhesion process. They are composed of two non-covalently linked polypeptide chains [for review see 48]. Three different subfamilies are defined by 3 different α chains. The β chain, or CD18, can associate with these 3 different chains (CD11-a-b-c) to constitute the LFA-1, Mac 1/CR3, and p150/95 molecules, respectively. These 3 molecules are expressed on leukocytes only, whereas their ligands, ICAM-1 (CD54), -2, and -3 for LFA-1, are widely distributed. ICAM molecules are present on endothelial vascular cells, macrophages, monocytes, and activated B lymphocytes [48]. ICAM-1 is up-regulated by TNFα, IL4, and IFNγ and can also be induced on fibroblasts, keratinocytes, and epithelial cells. LFA-1 was first defined by anti-CD11a mAbs, which inhibited cell-cell adhesion and several effector functions in vitro: cytotoxic T-lymphocyte (CTL)- and NK-mediated lysis, T-B cell cooperation leading to antibody production, and interactions between T/monocytes and T/vascular endothelial cells [51]. The cellular adhesion in which LFA-1 is involved enhances T-cell receptor/antigen recognition, and provides auxiliary signals for T-cell activation. T-cell activation also increases cell "adhesiveness" through conformational changes of the LFA-1 molecule. In humans, clinical studies using anti-LFA-1 have been conducted mostly in bone marrow transplantation. Congenital absence of LFA-1 is associated with a severe immune deficiency, suggesting that interfering with this molecule could also result in strong immunosuppression [54]. Fischer et al successfully used a mouse anti-CD11a (25–3; IgG1) in children who had undergone a bone-marrow transplant to prevent the rejection of HLA-mismatched marrow [55]. Another pilot study, however, failed to reproduce this result in leukemic adults receiving a T-cell-depleted bone marrow transplant, with the same mAb [56] or with an anti-CD18 mAb (β chain) [57]. The anti-LFA-1, mAb 25–3, also has been used in our center to attempt to reverse first-time acute rejection episodes in 7 recipients of a first-kidney transplant who were taking cyclosporine and azathioprine [6]. This anti-CD11a mAb, which produced only a few side effects, was not effective in 6 of the 7 patients treated, although circulating levels of mAb 25–3 were at concentrations 3- to 12-fold higher

than the mAb dissociation constant (Kd ~5 nM); this lack of effectiveness thus was not a consequence of low drug concentration. Interestingly, only one patient of the 7 developed anti-25–3 IgG at day 17, albeit low levels; this observation is consistent with experimental studies [58]. The low incidence of antibodies against mAb 25–3 might be related to the in-vitro ability of anti-CD11a to interfere in the cell-cell interactions leading to antibody production; interference with antibody production could be more sensitive to mAb 25–3 than the cellular events involved in acute rejection. Although attempts at treating acute cellular rejection episodes with anti-CD11a in renal transplantation have been unsuccessful, results obtained in bone marrow transplant recipients suggest that further studies might prove useful, particularly in prophylactic protocols.

Indeed, although inadequate in treating ongoing rejection crisis, the 25–3 anti-CD11 monoclonal antibody has been extremely encouraging when given prophylactically in patients receiving their first renal transplant. In a phase-I study of 15 patients receiving escalating doses (10 to 20 mg/day, administered for the 10 days following transplantation) in the absence of cyclosporine A, we have observed no rejection crisis during the first 30 days [59]. Administration of the anti-CD11a was associated with a full occupancy of the CD11a site, even when used at the lowest dose studied (10 mg/day) and with an impaired adhesiveness of the patient's T-cells for Daudi B cell line (ICAM-1^+) cells (Fig. 1). In addition, disappearance of the bright component of the classical bimodal membrane expression (bright/dim) was found at the end of the treatment, indicating CD11a (as well as CD18) membrane modulation. Only 3 of 15 patients were immunized, and the anti-mouse titers were remarkably low [59].

A mouse IgG2 antibody directed against one of the three ligands of LFA-1 (ICAM-1), R6.5, also has been tested in kidney and heart transplants; in cynomolgus monkeys, R6.5 delayed and reversed acute interstitial rejection [60]. In this study, the anti-ICAM-1 monoclonal antibody triggered an immune response in the primate and, although the renal endothelial cells of the treated animals were covered by the mAb, graft function was unimpaired and histologic examination did not show vasculitis. The same mAb was recently used in a pilot study in human and hyperimmunized recipients, or in patients having received a kidney with a long ischemia time. Although the number of patients in whom the mAb reached significant circulating levels was low, the incidence of acute rejection (2/7), as well as of delayed graft function, suggests that the interaction with either the receptor or ligand of the LFA-1/ICAM couple prevents allograft rejection [61]. Would the combined use of anti-LFA-1 and anti-ICAM induce a tolerance state, as in the mouse model [16]?

Despite these promising pilot studies, clinicians fear the potential for severe infection, and only larger trials will allow a more definitive conclusion. Clearly, these extended trials also will have to verify whether inhibiting the relevant epitope involved in ICAM binding to LFA-1, and therefore its interaction with all of its 3 ligands, will be a better strategy than interfering with ICAM-1, which is only one of the redundant ligands of LFA-1. Theoretically, both anti-LFA-1 and anti-ICAM-1 should equally prevent ischemia-related damage unless a further redundancy of an unknown LFA-1-ligand surrogate exists. A possible effect on delayed function in the anti-CD11a pilot study might have been missed in our trial, owing to the fact that this effect, suggested by the first pilot study with anti-ICAM-1, was noted only in recipients with long ischemia time, whereas short ischemia time was common in our study. Interestingly, a recent report, demonstrating that inhibiting another adhesion molecule (P-selectin) decreases white-blood-cell-related lesions [52], suggests a need for a deeper exploration of the effect on ischemia-related graft damage of antibodies (or soluble ligands) interacting with various adhesion molecules.

Another potential adhesion/activation target is the CD2. Antibodies have been successfully tested in vivo [53], and a clinical trial in which anti-CD2 antibodies are associated with anti-CD11a is currently underway in the prevention of mismatched bone-marrow grafts (Fisher A, personal communication). A 50–55 kD glycoprotein expressed by mature T-lymphocytes and NK cells in humans, CD2 contributes to increased T-cell adhesiveness to LFA3-bearing cells. The CD2/LFA3 interaction also provides co-signals that increase the antigen-specific signal, as shown by bispecific antibodies or by the use of a combination of certain anti-CD2 mAbs in vitro [62]. Futhermore, activation through CD2 increases LFAl-ICAM interaction [63]. In animals, anti-CD2 mAbs produced cell unresponsiveness and prolonged pancreatic islet grafts (both in naïve and immunized recipients) [64] and cardiac allografts [53]. Furthermore, a combination of an anti-CD2 and anti-CD3 results in the induction of a tolerance state [65]. A fusion protein between LFA3 and the constant part of IgG heavy chain can prolong allograft transplantation in monkeys through a mechanism that involves $CD16^+$ cells (Hochman PS, Chisholm P, Marboe CC, et al, unpublished observations). Thus, experimental evidence clearly shows that blockade of the CD2-LFA3 couple is promising in immuno-intervention.

Antibodies against co-stimulatory and co-receptor molecules

T-cells recognize "antigen" as peptides originating from the processing of intracellular (presented by class-I MHC molecules) or from membranous and internalized exogenous structures (presented by class-II MHC molecules). Optimal T-cell receptor interaction with the HLA/peptide complex (direct or indirect presentation of graft antigens) requires, however, the presence of other families of molecules [see 66 for review]. These families include co-receptor molecules, CD4 and CD8, which bind to different domains of class II and on an epitope of the 3 region of class I, respectively; adhesion molecules (such as LFA-1); and "second signal" molecules, such as CD28 or CTLA-4 [67]. All these molecules interact with their corresponding specific ligands at the membrane of presenting cells (MHC, ICAMs, and B7, respectively). Their presence can result in a restricted recognition (CD4 for class-II, and CD8 for class-I presentation, for instance), in increased cell/cell adhesiveness and avidity (LFA-1, for instance), and in the production of transcription factors for a variety of cytokines. These cytokines in turn will further induce a self and paracrine promotion of genetically committed T-lymphocytes, as for instance after interaction between B7 and CD8-CTLA-4 [67]. Other molecules are more specialized in T-helper–B-cell interactions, such as CD5 (on T-helper cells), which interacts with CD72, a specific B-cell ligand [50]. However, since the engagement of both co-receptors and adhesion molecules with their ligands have been shown to deliver co-stimulatory signals per se, and since "second signal molecules" such as CD28 do require cell/cell contact for transcriptional activation, the restricted terminology commonly used oversimplifies their dual effect, adhesion as well as stimulation.

The understanding of the various steps controlling T-cell activation opened new possibilities for immuno-intervention through the use of specific inhibitory monoclonal antibodies. Indeed, an antigen-presenting cell that does not express one of these molecules or that interacts with committed T-cells in the presence of a blocking antibody (which interferes with one of these receptor/ligand couples) will not optimally present antigen. In addition, new molecular tools such as truncated receptors or ligands (soluble CTLA-4 or ICAM-1, for instance) fused (or not) with the constant part of an IgG heavy chain have been successfully used and thus may represent alternatives to monoclonal antibodies [68]. But although the possibility of interfering with immune responses through the inhibition of accessory molecules has been actively explored in animals, only anti-CD4 has been used in clinical trials.

During antigen recognition by T-cell receptor, CD4 molecules on T-cells bind class-II molecules on presenting cells, increasing cell/cell interactions and delivering co-stimulatory signals. CD4 is a monomeric 55 kD glycoprotein that can associate with the $p56^{LcK}$ (a tyrosine protein kinase [69]); antibody-mediated cross-linking of CD4 results in increased tyrosine phosphorylation and kinase activity of P56LcK controlled by CD45 [70]. Engagement of CD4 before T-cell receptor cross-linking also can result in cell death by apoptosis. Several CD4 mAbs are available in various species. Encouraging results have emerged from their use in manipulating models of experimental organ transplantation in rodents and primates. In rodents, anti-CD4 mAbs given before grafting induced a state of donor-specific unresponsiveness [see 17 for review]. Second grafts from the same donor strain were prolonged, whereas acute rejection of a third-party graft was observed [71, 72]. Concomitant injection of soluble antigen and anti-CD4 mAb [or F(ab')2 fragments] results in a specific, long-lasting unresponsiveness to subsequent initial antigen challenges [73]. As donor-specific blood transfusions can also induce specific hyporesponsiveness to a subsequent organ allograft, combined treatment with donor antigen and anti-CD4 mAbs administered several weeks before transplantation has resulted in an indefinite acceptance of allografts in mice [17]. Induction of the unresponsiveness state was accomplished only at optimal donor-blood transfusion volume and mAb dosage. In this model, F(ab') fragments given at a high dose (times tenfold) also were effective, whereas perioperative injections of the mAbs were less effective than when administered weeks before grafting [17]. The mechanisms responsible for the tolerance induced by anti-CD4 mAbs remain unclear. More information is required regarding the relevance of various epitopes at the CD4 molecule recognized by the mAbs [74], as well as that of the depleting and/or modulating capacity of a given mAb. After administration of a large dose of a depleting antibody, the tolerance state that follows the nonspecific immunosuppression might result from the emergence of T-cells that have matured in a new environment (that is, in the presence of graft antigens), thus resulting in specific inactivation of a T-cell clone against the alloantigens. Furthermore, $CD4^+$ splenocytes from tolerant mice administered with an anti-CD4 mAb actively transfer the specific unresponsiveness state to naïve syngeneic animals [75]. Anti-CD4 mAbs also have been reported to be poorly immunogenic; this finding suggests that they could induce self-tolerance [58].

Post-graft treatment by OKT4A or a mixture of two anti-CD4 mAbs (OKT4 and OKT4A) in rhesus monkeys prolongs renal graft survival [76]. Several preliminary reports in humans have appeared; the first study used BL4 antibody (IgG2a) administered from day 3 to 14 after transplantation [77]. Although this series was too small to allow any definitive conclusions regarding BL4's effect on early rejection (4 episodes in 12 patients), only one patient developed anti-BL4 antibodies; some anti-CD4 antibodies probably thus can mimic in humans what has been observed in mice [58]. A non-depleting, non-modulating IgG2a, OKT4A was used prophylactically (8–12 days at 0.2 mg/kg/day) in 6 patients, along with a triple induction protocol. Although all patients experienced acute rejection within 6 weeks (3 during OKT4A treatment), no firm conclusion can be drawn because the dosage and timing of OKT4A were not pharmacokinetically optimal [78]. Interestingly, whereas this dose of the mAb was highly immunogenic in humans, a humanized chimeric OKT4A (IgG4) triggers no reactivity in non-human primates [79].

We have just completed a study of a murine IgGl directed against the second domain of CD4; the antibody was administered in 14 patients for 10 days after renal transplantation (unpublished observations). This mAb transiently depletes $CD4^+$ cells and modulates CD4 molecules at the cell membrane. The mAb was not associated with any anti-CD3-like side effects and did not produce a tolerance state; 4 patients experienced acute interstitial rejection shortly after cessation of the mAb. Thus, although the use of blocking, or transiently depleting, anti-CD4 might be somewhat effective and possibly introduces a level of specificity in the treatment of graft recipients, preliminary experience does not suggest that tolerance is achieved in monkeys or humans following anti-CD4 administration. The data are far from conclusive, however. For instance, clinical trials are restricted to administration of anti-CD4 shortly after transplantation or immediately postoperatively; these conditions favor nonspecific immunosuppression rather than specific tolerance. In experimental animals, donor-specific suppression occurs only several weeks or months after anti-CD4 administration [17].

In addition, immuno-intervention through CD4 manipulation is confounded by hazards inherent to the complexity of mechanisms of the CD4-mediated effect in allorecognition. First, the respective involvement of CD4 versus CD8 populations depends highly on the expression of either class-I or -II MHC molecules on the graft, the pre-immunized or naïve status of the recipients, and the kinds of grafted tissue; inhibition of the $CD4^+$ subset thus might not always be adequate. Our current understanding of the mechanisms of action of anti-CD4 is probably simplistic. We perceive a decrease in the affinity of the $CD4^+$ cell in immune interaction that results from the administration of blocking, modulating, or depleting anti-CD4, and a subsequent impairment in helper T-cell function. But peripheral blood depletion is not always associated with a significant lymph node or thymic depletion [17]. Furthermore, in mice treated by an anti-CD4, a peripheral anergy has been evidenced in alloreactive cells identified by monoclonal antibodies ($V\beta5^+$ $V\beta11^+$); however, the co-administration of anti-CD8 prevented the development of anergy of $V\beta5$ $V\beta11$ cells without blocking the tolerance against the allogeneic islet cells used in this model [80]. Also, helper memory cells and the TH2 population producing IL10 and IL4 escape anti-CD4 therapy in rodents [81], whereas only few IL2-producing cells were found. This profile suggests an imbalance in TH1/TH2 reminiscent of that reported in donor-cell-injected cardiac graft recipients [82]. Although CD4 manipulation is extremely promising, we need to

further understand its mechanism of action and its role in the treatment of patients undergoing renal transplantation.

Monoclonal antibodies against interleukin 2 receptors

The availability of antibodies directed at activation determinants induced on the genetically pre-committed lymphocyte subset, such as mAbs targeting IL2-receptor (IL2R), offers a new possibility for more selective immunosuppression. Resting T-cells neither express the α(P55) low-affinity component of IL2R nor the functional high-affinity IL2R. Following antigenic stimulation through the T-cell receptor, specific T-lymphocytes are activated, IL2 is secreted, and functional IL2R is transiently expressed [83]; this process allows antigen-committed lymphocytes to expand. At least three polypeptide chains contribute to the formation of the functional IL2R. The α or Tac chain was the first component recognized; this 55 kD glycoprotein is the antigen-inducible structure of the system and, after antigenic stimulation, its expression increases by at least 10 times. This chain alone has a low affinity for IL2 (Kd 20 nM) [83] and is not able to internalize IL2 [84]. The 75 kD β chain has an intermediate affinity for IL2 and is required to internalize IL2. The β chain, needed to optimize signal transduction, is also present on resting T-lymphocytes and natural killer cells. After antigenic α chain induction, approximately 10% of α chains associate with the β chain and form the high-affinity IL2R (Kd 30 pM) [85] in the presence of a third component, the recently characterized gamma chain [86]. The gamma chain is involved in the high-affinity conformation as well as in the signal transduction; specific mutations of this gamma chain, which is shared by IL4 and IL7 receptors [87], result in severe congenital immunodeficiency in humans [88].

As P55 contributes to the high-affinity complex, mAbs directed at the IL2-binding site of the α chain are potent inhibitors of IL2-driven proliferation. Their presence, in sufficient amounts to block IL2-binding, therefore should result in the specific inhibition of the growth of the pre-committed recipient cells activated by foreign antigens. Theoretically, this approach spares the resting T-cell repertoire. But the recipient's immune response against any other T-cell-dependent antigens introduced into the recipient during anti-IL2R treatment also will be depressed. Interleukin 2 has been well documented as a major pivotal growth factor for T-lymphocytes; however, it is likely that other lymphokines can substitute for IL2. Verification comes from studies of animals in which the IL2 gene has been disrupted by homologous recombination [89] and which can still mount some T-cell response. Both IL4 and IL7 are potent T-cell growth factors that can partially replace IL2. However, antibodies directed against IL2R are highly effective in a variety of animal models and in humans. In the mouse, treatment for only 10 days produces indefinite graft survival in more than 50% of recipients [90]. In the rat, anti-IL2R antibodies are effective as single agents and also act in synergy with cyclosporine [91]. A mice anti-human α chain of IL2R (anti-Tac) allows the prolongation of renal allografts in cynomolgus monkeys [92].

We have used a rat IgG2a mAb (33B3.1), which inhibits IL2 binding on both P55 and high-affinity IL2R, and which blocks IL2-driven proliferation [93, 94], to determine its effect in kidney transplant recipients [95]. To date, we have given this mAb to 135 recipients of first or second renal or renal/pancreas transplants. Almost all recipients tolerated the 33B3.1 well. Although a dose of 5 mg/day was insufficient to obtain "therapeutic" trough levels, 10 mg/day produced a trough level of approximately 4 μg/ml and gave encouraging results in a preliminary study on the prevention of acute rejection episodes [95]. We gave the mAb immediately after transplantation and for 2 weeks thereafter along with corticosteroids and azathioprine.

We subsequently conducted a prospective, randomized, controlled trial [96]. One hundred consecutive recipients of primary cadaveric renal transplants received either 33B3.1 (n = 50) or a rabbit ALG (n = 50). The 33B3.1 was given at 10 mg/day in the first 2 weeks after surgery with 1 mg/kg of methylprednisolone and 2 mg/kg of azathioprine; at the end of the mAb course (day 14), CsA was started. Only one patient treated with 33B3.1 required that the drug be stopped because of major clinical intolerance, versus 16 (32%) patients in the ATG group. A similar number of rejection episodes occurred during the first 3 months (15 versus 12 in the 33B3.1 and ALG groups, respectively). However, the 33B3.1-treated recipients experienced more rejection episodes during the first 2 weeks (6 versus 1). Between months 4 and 12 after transplantation, 7 patients in the ALG-treated group experienced a total of 9 rejection episodes versus 3 patients with 3 rejection episodes in the mAb group (NS). After the first year, the incidence of rejection episodes was 5% in both groups of patients. Fewer episodes of infectious disease occurred in the 33B3.1 group (47 versus 72); possibly the activated T-cell specificity produced more specific immunosuppression. Regarding viral infections (in which T and NK cells have been mostly involved), 8 of the 9 cases of cytomegalovirus disease in the mAb group were mild, while half (5 of 10) were severe in the ALG-treated group. After three months, the number of infectious episodes remained lower in patients who received the mAb as compared with those treated with ALG (9 versus 23). A strong immunization against rat IgG2a occurred in 80% of patients receiving 33B3.1, thus significantly lowering circulating mAb levels.

Less-favorable results have been obtained in two additional studies conducted in repeat renal transplantation [97] and in simultaneous renal/pancreatic transplantation [98]. In second renal transplant recipients, 33B3.1 was given for the first 10 postoperative days in association with steroids and azathioprine (CsA was started at day 10). Similar patient and graft survival rates were observed at one year (77% versus 71% in ALG and 33B3.1), and an equal incidence of rejection episodes occurred with both regimens (45% and 44%). Rejection was more frequent (40% versus 0% in the ALG group) during the 10 days of therapy with the monoclonal antibody, however, despite high circulating trough levels. Almost all recipients were sensitized by 33B3.1. In double graft recipients, CSA was associated with 33B3.1 for the first 10 days. No rejection occurred during the 10 days of treatment in these patients. Later, episodes of kidney rejection were more frequent in the 33B3.1-treated patients. All these diabetic recipients had received grafts without tissue matching. Patient survival was 83% and 100% in the ATG and 33B3.1 groups, respectively.

Because the 33B3.1 was effective in preventing rejection episodes and anti-IL2R could reverse ongoing rejection in animals, we initiated a pilot study to assess the effect of this mAb in the treatment of 10 initial, acute interstitial rejection episodes in first kidney grafts [5]. Six episodes partially responded to treatment and 4 required rescue treatment. During ongoing rejection episodes, the effector T-cells had already proliferated, expanded within the transplant, and recruited inflammatory cells. At that

time the effector mechanisms resulting in rejection are no longer restricted to activated T-lymphocytes, which are the primary targets of anti-IL2R.

Other clinical trials have been performed following renal transplantation with different anti-P55 mAbs. Kirkman and colleagues reported a randomized trial of anti-Tac (murine IgG2a against P55 chain) in human renal transplantation [99]. As we did, they gave anti-Tac mAb as induction therapy along with steroids and azathioprine, but CsA also was added. Their results suggest that anti-Tac decreases early (first month) rejection episodes in renal allografts. Other studies have shown the efficacy of a rat mAb, IgG2b (LO-Tac-1), and a mouse IgG1 mAb (BT563), in renal and liver transplantation [100, 101]. The literature appears to confirm that blocking IL2 binding results in successful allotransplantation. But it is too early for us to conclude that this approach also will be beneficial in transplants performed in hyperimmunized patients or on second grafts. The availability of "humanized" mAb [102], and the use of mAb combinations [103] or bispecific mAb [104] might further enhance the efficiency of anti-IL2R. Nevertheless, anti-IL2R currently is probably the most promising bioreagent in prophylactic therapy for first-kidney grafts, owing to its absence of side effects and higher specificity.

Antibodies directed against cytokines themselves also have been tested in animals. The theoretical advantage of such an approach would be high biologic specificity; however, the local range of activity of most of the cytokines, and the stoichiometric ratio of ligand/receptor, might explain the dearth of reports.

Other targets, monoclonal antibody combinations, and engineered reagents

A variety of monoclonal antibodies directed at epitopes present on resting or activated T-cells—or with wider distribution—also has been used experimentally and sometimes clinically. These mAbs belong to a heterogeneous group including anti-CD52, anti-CD45, and antibodies directed against yet-unknown specificities.

Campath-1. The family of Campath-1 antibodies comprises rat mAbs of various isotypes, including IgM as well as a chimeric human IgG. These mAbs recognize a phosphatidylinositol-anchored antigen (CD52) expressed on virtually all white blood cell lineages [105]. Campath-1 binds human complement [106] and effectively purges bone marrow of T-cells through human-complement-mediated cytotoxicity. Campath-1 has been evaluated further in a randomized trial in 52 renal allografts [107]. The protocol compared Campath-1 (25 mg/day for 10 days) and high doses of CsA (17 mg/kg) with high doses of CsA alone. The incidence of rejection episodes was lower in the mAb group; however, major infection episodes significantly increased after mAb treatment. The anti-rat immunoglobulin antibodies, detectable on day 10, further increased during the following 7 days.

Other IgM mAbs. Although the use of Campath-1 seems attractive when compared with polyclonal anti-lymphocyte globulins, this approach carries the risk of over-immunosuppression, a problem difficult to assess because the mAb was administered with high doses of CsA [106]. The effectiveness of this monoclonal antibody is likely related to its capacity to bind human complement. Thus IgM mAbs probably act more via their isotype-linked properties rather than by interacting with a molecule exclusively involved in alloimmune recognition.

Accordingly, several groups have followed the same approach of using a monoclonal antibody of the IgM isotype in an attempt to increase effector function. Takahashi et al first used the CBL1 mAb (a mouse IgM) directed against a determinant present on human lymphoblast cells [13]. They administered the CBL1 mAb at 5 mg/day for 9 days in acute steroid-resistant rejection episodes. The mAb was beneficial in 50% to 90% of the episodes [108]. Despite these promising initial studies, results concerning prophylactic utilization of CBL1 in allograft recipients of living-related kidneys were inconclusive [109].

Another IgM, the CHAL 1 monoclonal antibody (anti-T and -B cells and anti-monocytes), was tested at 12 to 24 mg daily doses over 9 days in 28 renal graft recipients undergoing acute corticosteroid-resistant rejection episodes (4 living-related grafts, 18 first, and 6 second cadaveric grafts) [108]. Fourteen cadaveric graft recipients received CsA and prednisone; the others received prednisone and azathioprine. The reversal rate was higher among CsA-treated patients than among azathioprine-treated patients (81% versus 50%), but 33% of the patients treated with CHAL 1 had rejection recurrence in this preliminary report [108].

Anti-CD45. Also of IgM isotype, this monoclonal antibody has been used to eliminate passenger leukocytes within allografts, particularly the interstitial cells of bone marrow origin, which are believed to play a major role in the "direct" pathway of presentation of donor-specific antigens to the recipient lymphocytes. This mAb is directed against a widely expressed CD45 isoform present on almost every white blood cell lineage (a protein tyrosine phosphatase that is produced as additional forms from a single gene by alternate splicing). Ex vivo, anti-CD45 treatment of renal grafts produces a high degree of coating of $CD45^+$ cells and significantly decreases the incidence of subsequent rejection (18% versus 63%) [110]. The availability of a monoclonal anti-pig CD45 has allowed the same group to better assess the ex-vivo perfusion conditions and reach a $CD45^+$ labeling rate of at least 95%.

Anti-CD7 mAb. Anti-CD7 monoclonal antibodies recognize a 40 kD antigen present on T-cells and preferentially on T-cell blasts [111]. A chimeric anti-CD7 mAb derived from the RFT2 hybridoma [9] has been studied as a prophylactic regimen in renal allograft patients. The mAb was well tolerated, induced a modulation of the CD7 molecule, and did not trigger anti-mAb host antibodies. Further, the mAb's ability to prevent early rejection was encouraging [10].

Monoclonal antibody combinations and bifunctional antibodies. Although combinations of mAbs recognizing synergistically active molecules have been shown to be more potent in inhibiting some immunologic functions in vitro than when used alone, in-vivo experiments have been limited so far. In recipients of mismatched bone marrow, a slight beneficial effect on rejection was observed in patients receiving anti-LFA-1 and an anti-CD2 (Fisher A, personal communication). The combination of anti-LFA-1 and anti-CD2 has proved highly effective in inhibiting mixed lymphocyte response (MRL) in vitro (Böhmig GA, personal communication). There are, in addition, several other examples in which combinations of two mAbs are attractive. For instance, 33B3.1 antibody cannot block 100% of IL2- (or mitogen)-induced proliferation of alloreactive clone in vitro, whereas when 33B3.1 is combined with a second mAb directed to IL2R β chain, the proliferation of the IL2-induced clone, as well as IL2 binding, are almost completely inhibited [103]. This finding suggests that the

combination would increase the effectiveness of anti-IL2R therapy. Interestingly, it is the combination of two antibodies directed at epitopes carried by independent targets such as ICAM and CD11a [16] or CD2 and CD3 [65] that has been able to achieve tolerance in animals, whereas the administration of a single mAb was ineffective.

Another way of combining the effect of two mAbs displaying potentially synergistic functions is preparing bispecific antibodies. Keeping with the IL2R targeting example, bispecific antibodies carrying both anti-P55 and anti-P75 specificities are highly efficient in inhibiting IL2 binding and IL2-dependent proliferation in vitro [104]. Although it is difficult to conceive that clinicians will soon have the opportunity to demonstrate as clinically valuable all the theoretical possibilities of intervention using mAbs, bifunctional antibodies also could be used to increase the targeting efficacy to a given molecule if one of the specificities is devoted to CD3 recognition [112]. This dual recognition will result in the killing of cells that have been brought into the $CD3^+$ cell vicinity by the second specificity of the bifunctional killing.

Conclusion

Although only partially explored in animals and having restricted application in humans so far, the use of monoclonal antibodies in transplantation has opened up a large field of new possibilities. However, the high clinical cost of this approach will no doubt limit the study of many mAbs. In many instances (for example, anti-CD4) different results may arise from the use of antibodies that recognize distinct epitopes on the same molecule; this situation will render unpredictable the effect of some mAbs in humans. Furthermore, some degree of the species specificity in the immune response (as well as variations in the pattern of target distribution between humans and animals) can make even more difficult the extrapolation of the usefulness of an antibody active in animals to the clinical setting. Finally, the paradigm of an exclusively specific intervention on the immune system is tempered in practice by the role of the constant parts of the antibodies—and of their corresponding isotypes—that will be even more important in "humanized" reagents. Indeed, "optimal" constant part-related effector functions (for example, complement-fixing isotypes) can result in cell destruction with a decreased specificity of action. Therefore, working hypotheses based on specific targeting of key molecules at the membrane of immunocompetent cells might be fundamentally biased except for targets expressed on highly restricted cell subsets (such as the IL2 receptor). For target molecules more widely expressed, effector mechanisms aimed at a specific intervention should work through membrane modulation of the molecule or specific competition with the corresponding ligands. Although molecular engineering of the mAb can favor either alternative—strict molecular target specificity by the use of scFv fragments for instance [47], or "optimal" constant region by the choice of the adequate isotype—the final relevance of the "target effect" still depends on all these factors.

Questions and answers

DR. NICOLAOS E. MADIAS (*Chief, Division of Nephrology, New England Medical Center, Boston, Massachusetts*): What is the best available explanation for the marked release of cytokines after OKT3 administration but not after treatment with other anti-T-cell mAbs? Is it a difference in cytolysis or in T-cell activation?

DR. SOULILLOU: It seems that the magnitude of the side effect in vivo correlates with the proliferative signal delivered in vitro by the anti-CD3. The differences likely are due to the isotypes of the mAb that control Fc-related functions. The in-vivo side effect will then depend on the capacity of the mAb to bridge the monocytes and macrophages that activate T-cells. Some recipients have fewer side effects than do others because they express Fc-receptor polymorphism. Some Fc species do not interact with Ig1 or Ig2b mAb for instance [113]. In this last case the magnitude of side effects varies with individuals.

DR. MADIAS: In describing your clinical trials using anti-LFA-1 or anti-IL2R mAbs, you emphasized that you avoided the co-administration of cyclosporine A. Was this done primarily to aid in the interpretation of the data or to potentially prevent the possible selection by lymphocytes of an alternative growth pathway to IL2?

DR. SOULILLOU: In first grafts, almost 100% of acute first rejection episodes that might occur despite mAb treatment would have been reversed by steroids and ATG, if needed. Therefore, CsA was not given with the tested reagent because the risk of over-immunosuppression from CsA appeared to us potentially more dangerous than that of a rejection episode.

DR. ANDREW KING (*Division of Nephrology, New England Medical Center*): Could you comment on the impact of cold ischemia on expression of ICAM in the endothelial cells of the grafts, and whether that can have an impact on blockade of LFA? In your original trial, did you note any relationship of cold ischemia time to the incidence of rejection?

DR. SOULILLOU: We have not studied the effect of ischemia on ICAM-1 expression. However, in contrast to ICAM-1, LFA-1 expression is not regulated by cytokines. I see no reason why an increased expression of ICAM-1 molecules will affect the LFA-1 inhibition by the mAb. In fact, the unregulated LFA-1 expression is probably an advantage of using anti-LFA-1 instead of anti-ICAM-1.

DR. MADIAS: Regarding the possible relationship between ICAM-1 and renal ischemia, I should mention the exciting preliminary data that Dr. Joseph Bonventre of the Massachusetts General Hospital presented to us recently at a research seminar. Administration of an anti-ICAM-1 antibody to rats at the time of ischemia or even 2 hours after the ischemic period resulted in marked functional and morphologic protection [114].

DR. SOULILLOU: Cosimi's group recently reported a preliminary study in Transplantation suggesting that anti-ICAM-1 prevents delayed graft function of transplants stored at 4°C for more than 48 hours [61]. However, only few patients with high enough anti-ICAM-1 trough levels were studied, and these results need confirmation. If this protective effect is related to the inhibition of leukocyte adhesion to ischemic endothelial cells by interfering with the ICAM-1/LFA-1 interaction, then blocking of LFA-1 should be even more effective because more ligands (ICAM-2, -3, and unknown) should be involved. As in our pilot study with the anti-CD11a, cold ischemia time longer than 48 hours was an exclusion criterion, and we have to wait for the result of a currently running randomized study to assess the effect of the inhibition of LFA-1/ICAM interactions on delayed graft function.

However, several reports now indicate that administration of soluble sugars, which are the ligands of selectin involved in the first step of the interaction of leukocytes with endothelial cells, is

effective in decreasing the inflammatory lesions on tissue microvasculature [52]. Similarily, inhibition of IL-8, which also indirectly controls the adhesion process, decreases the reperfusion syndrome [115]. Inhibition of LFA-1/ICAM-1 interaction, which comes later in this cascade, is probably also effective. I am personally convinced that the inhibition of adhesion molecules (integrins, L, E, or P selectins) or their ligands will be important in the future.

DR. JOHN T. HARRINGTON (*Chief of Medicine, Newton-Wellesley Hospital, Newton, Massachusetts*): I have two questions. First, does standard ATG have an effect on any of the targets that you discussed today? Second, can you expand on the term "immunologic correlates"? What specific tests would you use in monitoring renal transplant recipients? You mentioned the site occupancy of LFA1. What other tests might be employed?

DR. SOULILLOU: Anti-thymocyte globulin contains antibodies against a variety of targets [116]. However, I know of no evidence for attributing its effect to a single specificity. In this respect it is possible to consider ATG a "cocktail" of various mAbs.

The second part of your question relates to the monitoring of the effects of such bioreagents. Clearly, one cannot monitor accurately all the effects of ATG, although some of its effect—for instance, inhibition of ^{AET}T-cell rosetting—is of great practical importance [117]. But monoclonal antibodies usually have the advantage of allowing monitoring based on specific parameters. However, the relevance of such monitoring is confirmed only by the accumulation of clinical correlations. The monitoring can indicate a state of insufficient immunosuppression that is sometimes related to the host immune response, for instance, against the murine antibodies. For example, in our pilot study of the anti-LFA-1 trough levels, inhibition of conjugates between patient T-cells and DAUDI cells, CD11a and CD18 surface modulation, and CD11a site occupancy were compared with each other. The site occupancy test, technically the simplest, had a good correlation with the others (Le Mauff B, personal communication). However, only a large clinical experience will ascertain the relevance of this test.

DR. AJAY SINGH (*Division of Nephrology, New England Medical Center*): I would like your comments on two issues. The first issue regards chronic rejection. Brenner and coworkers have suggested that nephron number and hyperfiltration, rather than immunologic factors, are key factors in the pathogenesis of chronic rejection [118]. Do you agree with this view? Furthermore if, as I suspect, your view supports an immunologic basis for the syndrome, what is the status of research of relevant targets in chronic rejection?

My second question relates to the immunologic phenomenon denoted by the term "modulation," that is, endocytosis of cell-surface markers. Although modulation of CD3 secondary to OKT3 has been documented [27, 28], it does not appear to be of clinical significance because T-cells without CD3 are immunologically ineffective. However, does anti-CD4 therapy cause modulation of the CD4 molecule? Furthermore, could the limited benefit of anti-CD4 therapy be related to modulation?

DR. SOULILLOU: Dr. Brenner's hypothesis is attractive, although I think that "in addition to" would be wiser than "rather than," because there is a large body of information on the importance of immune mechanisms in chronic rejection; for instance, fully matched kidneys have less frequent chronic rejection. The precise immunologic mechanisms of chronic rejection and their relevant targets are yet totally unknown. However, decreasing the incidence of early acute rejection by an efficient prophylactic treatment might be a way of decreasing the incidence of chronic rejection.

Regarding your question on modulation, I do not agree that the CD3 modulation is not a relevant test because $CD3^-$ cells are "immunologically ineffective." Indeed, it is that test that tells you that $CD3^-$ has been modulated. It seems to me that a test that tells us that T-cell function is impaired during OKT3 treatment is important in many circumstances, particularly when neutralizing antibodies are suspected. However, better information could come from analysis of cells infiltrating that graft during anti-CD3 treatment.

Regarding the anti-CD4 effect, some anti-CD4 mAbs cause modulation, but not all. That the effectiveness of an anti-CD4 correlates with its capacity to modulate anti-CD4 at the lymphocyte membrane would appear logical. However, K. Wood, of Oxford, presented preliminary evidence at the September 1993 Basic Science Symposium in Transplantation that, in the murine model, the capacity of CD4 modulation of an anti-CD4 mAb might not be an advantage for induction of tolerance, but rather that modulation might be a "defense" mechanism in this particular case. More information is needed.

DR. BHARAT V. SHAH (*Head, Nephrology Section, P.D. Hinduja National Hospital and Medical Research Centre, Bombay, India*): Did I hear you correctly that monoclonal antibodies to adhesion molecules are effective in preventing rejection but not as effective in treating an acute rejection episode? If that is the case, what is the explanation?

DR. SOULILLOU: Yes, the use of the same mAb, administered in sufficient amounts, was not able to reverse ongoing rejection in human recipients [5]. LFA-1 is always expressed on leukocytes. As LFA-1/ICAM-1 interaction is involved in adhesion between lymphocytes and endothelial cells, interfering with this interaction immediately (or even before grafting) impairs a major step of allorecognition. During rejection, effector mechanisms are multiple, and interfering with a single function might not be enough. If confirmed, the effectiveness of anti-LFA-1 given prophylactically, contrasting with its absence of effect in ongoing rejection, would suggest that it acts mostly at the level of trafficking of the cells into the graft. Interestingly, experimentally tolerant mice treated with a mixture of anti-LFA-1 and anti-ICAM-1 harbor no cellular infiltrate [15], a pattern opposite to that observed in donor-specific blood-induced tolerance, for instance [14].

DR. MADIAS: Does the degree of pre-sensitization of the recipient affect the responsiveness to immune intervention with the mAbs you described? Is there a difference in the development of host immunization against these mAbs between clinical responders versus nonresponders?

DR. SOULILLOU: We observed no correlation between the presence, or the level, of anti-panel reactive antibodies and the immune response of the host against the murine antibodies in the anti-IL2R study in which hyperimmunized patients were included. However, as hyperimmunized patients were excluded from the anti-LFA-1 study, I cannot answer your question.

DR. MADIAS: Excluding ICAM-1, are the other adhesion molecules you mentioned expressed on endothelial cells in the basal state?

DR. SOULILLOU: Regulation involves mostly ICAM-1. ICAM-2 is constitutively expressed at a high level on resting endothelial

cells and is not augmented by activation [119]; in contrast, ICAM-1 is only weakly expressed on resting endothelial cells.

DR. MADIAS: Do LFA-1(+) or IL2R(+) lymphocytes remain in the circulation during treatment with the corresponding mAbs? For how long after discontinuation of therapy do the mAbs remain in the recipient sera?

DR. SOULILLOU: Concerning studies with the anti-IL2 receptor, only about 1% to 2% of circulating cells expressing P55 (α chain of IL2R) could be found during the treatment [96]. However, it is difficult to ascertain that the mAb was the cause of this because the concomitant administration of steroids is lymphopenic. Highly sensitive methods of cytofluorometry performed in recipients of another anti-P55 mAb does not suggest that the mAb was either depleting or modulating (Amlot P, personal communication). Clearly, the situation can change with the mAb.

Regarding the second part of your question, in anti-IL2R treatment, no circulating mAb was found 1 to 2 days after cessation of treatment. In patients who had received anti-CD11a, the circulating mAb was present much longer; in fact, significant trough levels were observed between days 4 and 10 after the end of the treatment.

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