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Monoclonal anti-IL2-receptor in organ transplantation

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Abstract. So far, only monoclonal antibodies directed at functional target molecules on lymphocyte surface membrane have been proven useful in preventing or reversing allograft rejection episodes. Antibodies directed against light chain (P55) of interleukin 2 (IL2) receptor and able to interfere with IL2 binding on the IL2-receptor in its high-affinity conformation (only expressed on activated T cells) are effective in various animal models and recently in a preventive protocol in human kidney graft recipients. Thus, IL2-receptor targeting emerges as a new therapeutic strategy involving only a small pool of progenitors committed against donor antigens. Furthermore, membranous or soluble forms of P55, as indicators of the presence of alloreactive clones in the graft or at the peripheral level, may offer new tools for monitoring the rejection process.

Key words: IL2-receptor - Monoclonal antibodies.

In the cyclosporin A (CyA) era, there are two major immunosuppressive strategies for graft recipients during the first few weeks following transplantation, the period of maximum risk. The first involves the use of strongly immunosuppressive bioreagents, such as antilymphocyte globulins (ALG) [3, 26, 43], or of monoclonal antibodies (Mabs) directed at functional targets [47, 61] on T-lymphocyte membranes. The second strategy calls for the immediate use of CyA alone or together with corticosteroids (CS) and/or azathioprine (AZA) [15, 27].

Although good results have been obtained with both strategies, randomized studies [25, 45] have favored the former strategy, which allows initial acute tubular necrosis (ATN) to be reduced by avoiding early CyA-mediated nephrotoxicity and ensures a low rate of early rejection episodes. The last advantage is the likely origin of the increasing popularity of the ALG/CyA sequential strategy in heart allografts [6]. Nevertheless, this approach is hampered by many side effects of both ALG [9] and OKT3 [47], the only Mab routinely used now. In addition, these bioreagents have the disadvantage of interfering with - or, in the case of ALG - of destroying all mature T-lymphocytes, whether or not they are involved in the rejection process. In contrast, Mabs that could only target activated cells actually involved in alloresponse and leave the remaining immune repertoire unimpaired would represent an important step toward specific immunosuppression. Among the many possible targets expressed only on activated alloreactive lymphocytes, antibodies (or modified ligands [30]) directed at the interleukin 2 (IL2) receptor (IL2-R) have been shown to be efficient in delaying or preventing early rejection in animal models, including the mouse heart [31] and skin allografts [20], as well as rat [35] and monkey kidney grafts [57]. Recently, a pilot study [61] and a randomized trial [8, 32] have been performed in human kidney allografts. The rationale, results, and discussion of this selective immunosuppression, as well as other possible clinical uses (i.e., peripheral monitoring, graft imaging), and ways in which it can be used to take advantage of the selective presence of IL2-R on activated cells will be reviewed in this paper.

Alloreactive T-lymphocyte clones and progenitors

T-lymphocytes (see [41] for review) infiltrating a rejecting graft are derived from a small pool of progenitors to recognize major histocompatibility complex (MHC) as well as against several minor histocompatibility antigens (many of which are yet undetermined in humans) [11, 19], and possible graft-tissue-specific structures [20] on graft cells. The minimum estimated size of this precursor pool, assessed by limiting dilutions and not taking into account antigens that have failed to express on stimulating feeder layer cells in the methods used, is only a small percentage ($< 1\%$) [55] of the peripheral blood lymphocytes from unstimulated individuals. Inhibition of this small pool of progenitors at their very

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early stage of activation would, therefore, be an ideal model of selective inhibition of graft recognition by the recipient immune system. Expansion of these alloreactive clone progenitors is under the control of two major events:

1. Antigen activation through alpha, beta T-cell receptors leading to transcription of a set of genes, including those coding for growth factor receptors, among which IL2 receptor plays a major functional role [28, 53], as well as those coding for specific ligands of some of these receptors, such as IL2 [67], IFN gamma, also regulating class II antigens [51, 64], and IL4 [50]. Although virtually all mature lymphocytes can enter this program, some operational dichotomy exists since a number of lymphocytes express IL2-R and produce IL2 (mostly CD4+, a "helper" T cell genetically committed to recognition of MHC class II antigens), whereas others are mainly restricted to IL2-R expression and require logistic "help" (ligand availability) for optimal growth (mostly CD8+, a cytotoxic T cell genetically committed to recognition of MHC class I antigens). CyA [33] and CS [18] act partly by inhibition of IL2 transcription, with selective inhibition of a cluster of growth factors that seem to be under the control of a common promoter [16].

2. IL2/IL2-R interaction (via the high-affinity IL2-R) then takes place and, although not unique as a growth-triggering signal, appears to be the major one initiating mitosis and clonal expansion of the progenitors.

Host-donor-driven T-lymphocyte expansion is, therefore, dependent upon this autocrine loop, involving only specifically activated antigen-committed lymphocytes. At day 7 after kidney graft, activated peripheral lymphocytes bearing Tac chain (R−IL2+) might be dramatically increased in unmodified monkey recipients [57].

IL2-receptors

IL2-R is composed of two major glycoproteins, P55 and P75. P55 (Tac chain), a 55 kDa glycoprotein [71] with low affinity for IL2 (K_D ~30 nM), was characterized and cloned first [38]; it is virtually absent from the surface of resting T-lymphocytes [53] and upregulated after antigen activation [28, 53]. High copy numbers (> 100,000) are expressed on alloreactive T-cell clones [28] extracted from rejecting grafts and stimulated with organ-donor Epstein-Barr virus (EBV)-transformed cells [44]. IL2-R expression is further regulated by IL2 itself [28], as well as by other cytokines [23]. However, IL2/P55 interaction per se does not appear to initiate internalization of the complex or trigger a mitogenic signal [73]. To obtain this effect, P55 must be combined with P75, a second IL2-R chain, which, when expressed alone, exhibits intermediate affinity for IL2 (K_D ~1 nM) [58, 59]. Although not sequenced, P75 is believed to have a larger intracellular domain than P55 since it is internalized after IL2 binding and can trigger entry into the S stage of the cell cycle and mitosis [70]. High-affinity IL2-R (K_D ~20 pM) requires the formation of a P55/IL2/P75 complex, which is internalized and then efficiently triggers cell division. At equilibrium, high-affinity receptors are estimated to be approximately 10% of the number of P55 copies present on activated alloreactive clones [28, 36]. High-affinity interaction of P55/P75 IL2-R with IL2 is allowed to occur by complementation of the rapid IL2 binding/dissociation properties of P55, with longer ones characterizing P75 [60]. Indirect evidence suggests that IL2/P55 binding takes place first and then anchors the P75 chain to constitute the functional complex. The availability of Mabs directed at P75 epitopes [65] will facilitate study of P75 expression. Thus far, IL2 binding and crosslinking experiments suggest that P75 is present at low density (< 1000 molecules/cell) on resting T-lymphocytes and is also upregulated, but to a lesser extent than P55, upon stimulation [59]. Although the definitive organizational complexity of IL2-R has not yet been fully assessed (P75 could be polymorphic [24] and other glycoproteins could be associated with the complex [14]), it is widely understood that antibodies directed at the "low-affinity" Tac chain can inhibit IL2 high-affinity interaction with its receptor since the IL2/Tac binding site is involved in this high-affinity conformation. It is also noteworthy that the Tac chain, or even the P75 chain, is not strictly limited to T-lymphocytes. B-lymphocytes [4], macrophages and monocytes [23], as well as glial cells [2], are also able to express low levels of IL2-R.

Finally, aside from its functional importance as an activation marker in T-lymphocyte clonal expansion, P55 may be of interest for its membrane expression levels in estimating the in vivo immune activation state. Moreover, a truncated, soluble form of P55, shed from activated lymphocytes [29], may represent an informative marker of the size of the activated lymphocyte pool in various immune activation states [66], including allograft rejections (O. Bouchot et al., submitted for publication; [10]).

Monoclonal antibodies against IL2-receptors in animal organ transplantation

Injection of exogenous recombinant IL2 in umodified or CyA-treated rat recipient allografts shortens graft survival time [49]. Mabs against Tac antigens of rats [48], mice [31], and humans (crossreactive with

monkeys) [46, 71] have been obtained by several groups. More recently, Mabs immunoprecipitating human P75 have also been produced [65], some of which inhibit IL2 binding and functional IL2/IL2-R interaction. However, only anti-P55 has thus far been used in vivo. Rat and mouse immune repertoires allow recognition of several human P55 epitopes [36, 46]. These epitope clusters (or Mab clusters) can be divided into at least two major clusters, according to their capacity to inhibit (or not inhibit) labeled IL2 binding and IL2-driven proliferation of high-affinity receptor bearing cells. Anti-P55 Mab (or polyclonal sera raised against Tac chain peptides [13]), as well as specific mutagenesis [54], have thus contributed to mapping putative sequences involved in IL2/P55 interaction. Obviously, as rat and mouse Mabs are not cytotoxic in the presence of human complement [although some can mediate antibody-dependent cell-mediated cytotoxicity (ADCC)], those reacting with IL2-binding sequence (cluster 1) are ideal candidates for in vivo testing. Kirkman et al. [31] first demonstrated, in histoincompatible mouse heart allografts, prolongation of graft survival associated with a decrease of graft infiltrating lymphocytes when M7/20 Mab was given immediately after transplantation. Similar results have been obtained in heart and kidney allografts in rats [35], as well as in skin grafts in mice, after the administration of low-grade radiation also to recipients [20]. More recently, a mouse anti-human P55 chain [71] was used successfully to increase kidney graft survival in monkeys [55]. Fewer reports deal with the capacity of anti-IL2-R Mab to reverse ongoing acute rejection [9], although this has been shown to be possible in rat allografts [35].

In almost all documented studies, only anti-P55 rat Mabs able to interact with functional IL2/IL2-R interaction or ^{125}I-IL2-binding assays have prolonged allograft survival [68]. However, there has been a puzzling case in monkeys [22] in which a Mab that clearly inhibited IL2-dependent T-cell growth was nevertheless ineffective. Moreover, some antibodies in cluster 2, such as ART65, are unable to exhibit an in vivo effect when given alone in large doses (250 μg/rat per day). They can nevertheless synergize at low doses of another anti-IL2-R Mab (ART18, cluster 1) when both Mabs are given at a low dose (25 μg/rat per day), one at which they are both ineffective alone [12]. These facts, which require further studies of the precise mapping of the epitope recognized (Mab crossinteraction in a binding study), may be difficult to reconcile with the simple model based on IL2-binding inhibition and may indicate yet undocumented negative signals delivered by the Mab per se to IL2-R-bearing cells, a situation reminiscent of activation or negative signals mediated by anti-CD2 [42] or anti-CD4 [52], according to the epitopes recognized. These "illogical results" may also be linked to isotype characteristics which, in some models, have been shown to be an important feature [5, 72].

Anti-IL2-R monoclonal antibody in human allografts

In humans, we have demonstrated in a pilot study [61] that a rat IgG2 Mab (33B3.1) belonging to cluster 1 [36, 46] was efficient in preventing early cellular rejection in kidney allograft recipients. In this pilot study, an 8% incidence of early rejection during 33B3.1 administration was observed when 10 mg/day 33B3.1 was used during the first 2 weeks after transplantation, as compared to a 67% incidence in the last "historical controls" treated with CS and AZA in the same center. Interestingly, virtually no side effects were noticed [7]. In contrast, a dose of 5 mg/day was found to be inefficient (three rejections in nine patients) with low circulating Mab levels (~0.5 μg/ml) [61], whereas 10 mg daily doses were associated with serum Mab concentrations that were about eight times higher. This difference was probably due to the required saturation of the aspecific IgG2a binding site. Such concentrations, which are 25 times higher than those required in vitro to displace S35-labelled IL2 from its high-affinity binding site [36], are probably able to inhibit in vivo IL2/IL2-R interaction completely. However, ligand concentrations in the organ microenvironment are impossible to assess, the concentration of Mab in the graft is low in the animal model (see below), and soluble circulating IL2-R is able to bind to anti-IL2-R Mab [29].

A randomized study of 33B3.1 versus ALG has recently confirmed 33B3.1 efficacy, with a rejection rate at 1 month roughly similar to that of ALG-treated patients [9]. However, ALG treatment was associated with a high rate of side effects, which resulted in withdrawal of the drug in 16 of 49 patients. In addition, the data suggested that infectious episodes in general, and urinary tract infections in particular, were fewer in anti-IL2-R-treated patients. Host immunization rates - both IgM and IgG - against 33B3.1 were high (86%) and usually associated with a drop in 33B3.1 circulating levels [63]. Indeed, five out of the six rejections which occurred during the Mab treatment period were associated with an early IgG and/or IgM anti-33B3.1 recipient response and a drop - sometimes to undetectable levels - in Mab serum concentration. This could suggest that, in the absence of an early host-anti-Mab response, anti-IL2-R may be fully protective. However, the drop in 33B3.1 level did not precede the rejection, and we therefore favor the hypothesis that the two phenomena are indicative of a "high-responder" phenotype without any direct relationship. In addition, as soluble Tac chain [29], consistently found at low concentration in recipient sera,

may increase during rejection [10], it may also counteract 33B3.1 effects by complex formation through idiotype interaction. Interestingly, soluble IL2-R (S-IL2-R) levels were always undetectable during 33B3.1 therapy. Circulating S-IL2-R also present a major drawback for the assessment of host anti-idiotypic antibodies since they behave as competing agents in the ELISA assay [37]. Recently, we eliminated S-IL2-R from recipient sera by ion exchange chromatography and were able to test them for the presence of host anti-idiotype antibodies in a competitive assay in which ELISA plates were coated with 33B3.1 complexed with soluble Tac chain. Chromatographed sera were added secondarily to peroxidase-labeled 33B3.1. Preliminary results indicate that, although detected in almost all immune sera tested, anti-idiotypic antibodies did not correlate with rejection episodes (B. Le Mauff et al., manuscript in preparation).

Neither IL2-R-bearing circulating lymphocytes nor circulating 33B3.1-bearing lymphocytes were detectable by flow cytometry using fluoresceinated anti-IL2-R Mab [59, 63], thus indicating opsonization of the target cells and/or an absence of IL2-driven expansion of alloreactive clones. Surprisingly, 33B3.1 treatment was associated with a 40% drop in circulating lymphocytes involving both CD4+ and CD8+ cells [7]. However, further studies of 33B3.1 administered to patients undergoing acute rejection episodes showed no lymphocyte drop [9]. As these patients were under CyA monotherapy, while those preventively treated also received CS and AZA, it would appear that the combination of CS and AZA was responsible for the lymphopenia. Recently, another anti-IL2-R Mab (anti-Tac, cluster 1) [71], a mouse IgG1 directed at an epitope closely related to that recognized by 33B3.1 and with similar K_D and K_I in the presence of ligand [36], has also been used in a prophylactic protocol in human kidney graft recipients [32]. In a short randomized series, anti-Tac (20 mg/day) added to CyA and CS significantly delayed the first rejection episode as compared to a regimen combining CyA and CS only. Preliminary data suggest to the authors that better results could be achieved with anti-Tac combined with low-dose CyA [32]. However, a larger recipient cohort is required to reach a firm conclusion.

Only a few patients undergoing acute cellular rejection episodes have thus far been treated with anti-IL2-R [9]. In this study, two out of ten episodes were reversed without using any other antirejection treatment. However, in four out of ten recipients, even though blood creatinine levels did not increase further under 33B3.1 treatment, the levels reverted to prerejection values after methylprednisolone (1 mg/kg) treatment. Finally, four rejection episodes did not respond to Mab therapy and required methylprednisolone pulses. These intermediate responses were not due to an early rise in recipient antibodies against 33B3.1 or to early low-circulating levels of the Mab since a dose of 20 mg/day for the first 2 days, followed by 10 mg/day for 8 consecutive days, achieved high (and sustained) 33B3.1 serum concentrations (~5 μg/ml as early as day 2 [9, 62]). Although this study did not include enough patients to be conclusive, it suggests that anti-IL2-R is not immediately effective once antidonor recipient clones have expanded. These results are compatible with the fact that IL2 concentration increases in the microenvironment during rejection. Moreover, 33B3.1 does not immediately interfere with cytotoxicity or other effector functions, but rather acts by inhibiting the clonal expansion that normally takes place early after allostimulation.

Mechanisms of action

Anti-IL2-R used in vivo are probably related to inhibition of functional IL2/IL2-R interaction, as previously discussed. Opsonization and sequestration of antigen-reactive cells (ARC) may also operate, although this is not suggested by the inefficacy of cluster 2 Mab in vivo [68]. A combination of two anti-IL2-R Mabs from clusters 1 and 2 (ART18 and ART65, respectively) in low dosages has, however, been reported to result in synergistic effect (described earlier) in which isotype characteristics of the Mabs may be involved [12]. Very little information about ADCC mediation is available with regard to these antibodies, which are not cytotoxic in vitro in the presence of human complement. Stunkel et al. (personal communication) found isotype-related ADCC capacity in a series of mouse anti-rat IL2-R Mab, but its relevance in vivo is unknown. In other models, antibodies with high ADCC potency and C1Q complement fraction binding capacity in vitro (mostly IgG1) were also found to be most potent in vivo [5].

Sparing of suppressor cells has been reported in rats treated with ART18, a cluster 1 anti-IL2-R, on the basis of a transfer experiment using splenocytes of anti-IL2-R-treated animals secondarily injected into a naive, syngeneic host [56]. However, several different immunological manipulations, such as blood transfusion [40], antibodies against MHC class I and II antigens [21], and CyA [34], have also led to a tolerance state that can be transferred to naive recipients by lymphocyte injection. Anti-IL2-R Mab might, therefore, be another example of induction of such a state of actively maintained tolerance, something which appears to be a common feature of rodent allografts in which acute destruction of the graft has been prevented.

Biodistribution studies of a mouse anti-rat IL2-R (OX39) injected into rejecting recipients have shown

that only a low percentage of the injected ^{125}I-OX39 was localized in the rejected graft at day 5 after transplantation [69]. At that time, around 70% of OX19 (10% of OX1)-positive cells invading the graft were IL2-R+ (A. Romaniuk and J. P. Soulillou, manuscript in preparation). However, a majority of OX39 molecules bound through the Fc fragment on Fc receptor-binding cells invading the graft as evidenced by a similar distribution of an isotype-matched control Mab. In contrast, ^{125}I-F(ab')2 fragments of OX39 located specifically in the allograft but not in the syngeneic graft, while control (F(ab')2 had a similar distribution in both allogeneic and syngeneic grafts [69]. The low concentration of anti-IL2-R antibodies in rejecting allografts may partly account for their low effectiveness in ongoing rejection and suggests that the preventive regimen may operate mainly on ARC circulating in the first days following transplantation.

Other putative anti-IL2-R uses in transplantation

The presence of IL2-R-bearing cells (10% of OX1$^+$ cells and 70% of OX19$^+$ cells) able to bind labeled antibodies in rejected organs can be detected by gamma scintigraphy in the early phase of rejection (day 5) but not in syngeneic grafts in which some OX1$^+$ cells are present but virtually no IL2-R positive lymphocytes are detected [69]. IL2-R expression, as a marker of alloactivation, might thus lead to a new means of rejection monitoring, particularly in heart rejection, where endomyocardial histology is still generally required. Furthermore, the truncated form of IL2-R (P55), which, in vitro, is produced upon antigeneic stimulation in amounts quantitatively related to those of the membranous form [29], increases during rejection episodes [10]. Thus, aside from its use as a target in the treatment of graft recipients, S-IL2-R might also be an interesting means of measuring the T-lymphocyte activation state in vivo.

Future IL2/IL2-R interaction manipulation

Taken together, the data accumulated to date in animals and humans leave little doubt that at least some Mabs - and perhaps all of cluster 1 - are efficient in preventing graft rejection and that IL2-R is, in fact, a major functional target in the modulation of alloreactivity. Theoretically, this approach should spare non-MHC-committed parts of the immune repertoire and minimize the incidence of infectious episodes. Whether this prediction will hold up is, however, uncertain since some micro-organisms (e.g., CMV) are inoculated with the graft. IL2/IL2-R interaction inhibition should, therefore, also operate at the CMV-committed clone level. Interestingly, CMV infectious episodes were not significantly fewer in 33B3.1-treated patients than in ATG-treated patients in the randomized study [8]. Moreover, if Mabs are useful in defining functional targets with which to interact at the lymphocyte surface membrane, such as strong immunogens, they are not likely to represent a suitable means of immunomodulation. "Humanized" antibody molecules, natural or genetically produced "anchor minus" soluble receptor molecules (mostly restricted to the P75 chain owing to its higher affinity for IL2), able to complex IL2 before ligand/receptor interaction, may also offer alternative tools in the future. Mutated IL2 molecules, still able to interact with P55 but not with P75, and therefore unable to transduce induction signals, have recently been obtained [17]. They represent a model for the development of antagonists that will benefit from precise knowledge of the effects of interactive peptides on IL2, P55 and P75, and from the tridimensional structure of the ligand/receptor complex. Finally, hybrid gene constructs, using IL2 itself as a carrier of toxins (diphtheria or *Pseudomonas*) to target only high-affinity-bearing cells, have recently been engineered [1, 39]. IL2 diphtheria toxin has already been shown to be extremely effective in vivo [30]. Although these compounds may not prevent host immunization against bacterial toxin determinants and will require careful pharmacotoxic evaluation since some nonlymphoid cells (such as glial cells [2]) bear IL2-R, when used at concentrations that target only high-affinity-bearing cells, they may lead to the development of powerful bioreagents.

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