Interleukin 2 receptor targeted therapy. A new approach to combat allograft rejection

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Despite the relative effectiveness of Cyclosporine (CsA) and prednisone in clinical organ transplantation, acute rejection is usually responsible for deterioration or loss of allograft function. Treatment of this complex and primarily T lymphocyte dependent response mandates additional therapy, most frequently high dose steroids or polyclonal anti-lymphocyte globulins. These powerful modalities, however, although often effective, may produce serious complications owing to the broad state of immunosuppression imposed. The adverse effects of CsA, primarily nephrotoxicity, also warrant a search for alternate therapeutic regimens to replace it or allow its administration in reduced dosage. Moreover, many individuals are precluded from ever receiving an allograft as they have become sensitized to a multiplicity of histocompatibility antigens and have developed high titers of circulating alloantibodies. In addition, despite a negative cross-match, a significant number of patients, particularly those who have received donor specific transfusions or have rejected a previous graft, experience accelerated rejection occurring within the first few days after transplantation. This fulminant process which includes a panoply of both cellular and humoral immune components is often difficult to control and may be refractory to conventional therapy. Unfortunately, the ideal goal of transplantation, imposition of donor specific suppression of the host immune system with simultaneous tolerance to the foreign graft, remains elusive.

Hopes have been raised by recent advances in hybridoma technology which has produced effective and reproducible biological immunosuppression with monoclonal antibodies (mAbs) as pharmacological tools. Indeed, mAbs directed against T cells or T cell subsets have been employed with considerable success both in experimental and clinical transplantation. Nonetheless, the use of antibodies broadly reactive with differentiation antigens on T lymphocytes does not solve the problems of side effects caused by general immunosuppression. An ideal therapeutic agent should target only those lymphocytes which participate in rejection of the donor graft without affecting physiological host immune surveillance and normal defense mechanisms. Theoretically, this goal could be achieved by «antigenic suicide», or by using the appropriate anti-idiotypic antibodies or mAbs against the antigen combining site of T cell receptors upon the donor graft.. However, because of the intense polymorphism of transplantation antigens and the vast genetic repertoire encoding for the T cell antigen receptor, achievement of such forms of specific immunosuppression, at least temporarily, is highly improbable.

Interleukin 2 Receptor: A Potential Target for Immunosuppressive Therapies

Interleukin 2 (IL-2), a 15kD sialoglycoprotein secreted by antigen/mitogen activated CD4+ (T helper) lymphocites plays a critical role in the proliferative expansion of T effector cells [1]. IL-2 exerts its growth promoting activity via interaction with stereospecific cell surface glycoprotein molecules, IL-2 receptors (IL-2R), that are expressed de novo by all activated but not resting T, certain activated B cells and macrophages [2]. IL-2R are heterogenous in terms of their binding characteristics; high and low affinity receptors have been identified [3]. The existence of two types of molecules that bind IL-2 with low (p55 subunit, beta chain) or intermediate (p75 subunit, alpha chain) affinities has been demonstrated. The association between these two molecules constitutes the high affinity IL-2R [4]. Only this binding triggers internalization of the IL-2/IL-2R complex which is prerequisite for clonal expansion and continued viability of activated T cells. Theoretically, IL-2R targeted therapy may interrupt the rejection cascade after transplantation and may produce a selective immune defect in allograft recipients (figure). The recent availability of mAbs against the p55 subunit of the high affinity IL-2R binding allowed to test this hypotheses in several species.

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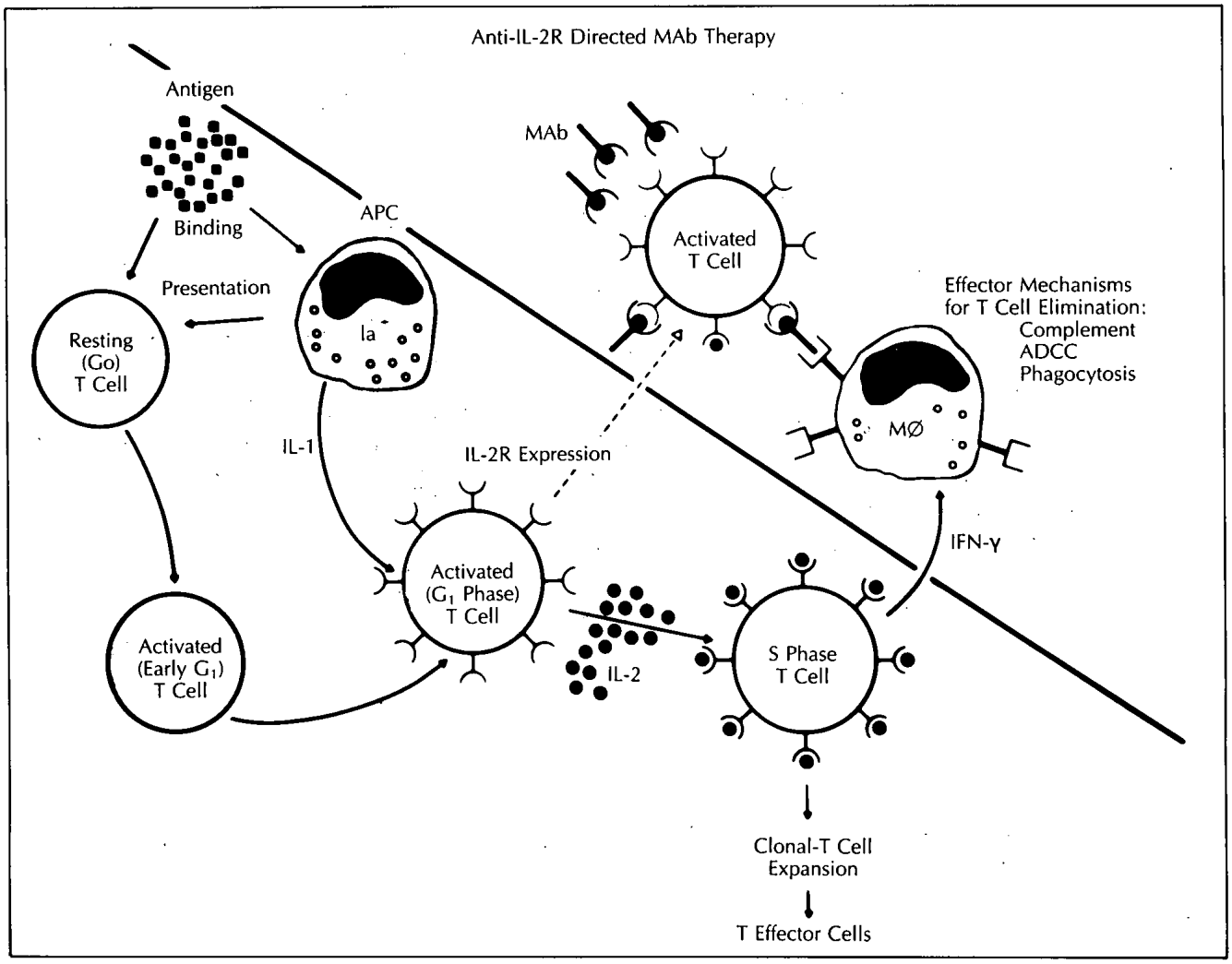

Fig. 1.—The pivotal role of IL-2R expression in cellular cascade leading to generation of T effector cells and a putative mechanism of action of anti-IL-2R mAbs in vitro are shown.
Abbreviations: ADCC: antibody dependent cell cytotosicity; APC: antigen presenting cell; IFN-γ: interferon gamma; IL-1: interleukin 1; IL-2: interleukin 2; IL-2R: interleukin 2 receptor; mAb: monoclonal antibody; MØ: macrophage.

Anti-IL-2R mAb Treatment in Organ Allograft Recipients

In this section we will first summarize the published reports on combating graft rejection in mice, primates and humans by focusing therapy selectively on IL-2R bearing cells. This will be followed by discussion of the complex interplay between anti-IL-2R mAbs and the host immune system in the rat models in which most of the in vivo experimental work has been done.

Organ Allografts in Mice: The first demonstration that IL-2R targeted therapy in organ transplantation was indeed effective, employed a rat M7/20 mAb (IgM) in murine recipients of heterotopic cardiac allografts [5]. Treatment with M7/20 (5 μg/mouse IP for 10 days after grafting) doubled graft survival in two inbred mice strain combinations; remarkably, a number of grafts survived indefinitely. Administration of mAb also reversed established rejection, as was evident from the groups in which treatment was delayed until day 3 or 6 [6]. Therapy with AMT-13, an antibody of the same specificity but of different isotype (IgG2a) was as operative as M7/20 [7]. Thus, the beneficial therapeutic effect achieved was a function of the IL-2R as a target and not necessarily of the mAb directed against it.

The effect of M7/20 on survival of skin allografts was also studied [6]. Although a 10-day mAb treatment resulted in a modest graft prolongation (ca. 15 days vs. 8 days in untreated mice), none of the skin grafts survived indefinitely. A low dose of x-irradiation (350R) as an adjunct to M7/20 therapy extended graft survival further to ca. 1 month [8].

Kidney Allografts in Primates: Murine anti-human p55 subunit IL-2R mAbs 1-HT4-4H3 and anti-Tac, both

of IgG2a isotype, react with Con A-activated cynomolgus monkey lymphocytes [9]. Both of these antibodies were tested in Macaca fascicularis renal allograft recipients [9, 10]. Administration of 1-HT4-4H3 had no effect on graft survival despite clearly demonstrable serum mouse antibody titers. In contrast, anti-Tac mAb infused as an IV bolus of 2 mg/kg into nephrectomized hosts preoperatively and every other day post-transplant until rejection occurred, was effective. Thus, the mean recipient survival in anti-Tac treated group was 19 days vs. 12 days in untreated recipients. Similarly, graft rejection as assessed by the rise of creatinine level (4 mg/dl), was postponed from day 6 to day 11 in control and anti-Tac treated animal groups, respectively.

The effect of anti-Tac treatment on the expression of IL-2R on the peripheral blood lymphocytes was also examined. In untreated hosts rejecting their grafts acutely, IL-2R+ cells were present at day 4-5 and peaked (25-40 %) on the first day of a rise in creatinine level. In contrast, in the treated group IL-2R expression peaked on day 12-13, i.e. preceeded the creatinine rise and subsequent allograft rejection.

It is highly probable that the loss of anti-Tac efficacy was associated with the development of anti-murine antibodies by the host. Indeed, nearly all monkeys treated with anti-Tac developed anti-mouse antibodies 7-10 days following initiation of therapy, the time anti-Tac has lost its ability to bind to IL-2R. Coincidentally, the time needed for the antibody development is almost the same as that of allograft prolongation following IL-2R targeted treatment.

Kidney Transplants in Humans: In Nantes and Boston, human recipients of cadaver donor renal allografts are receiving anti-IL-2R mAb as adjunctive immunosuppressive therapy. 33B3.1, a rat IgG2a antibody against IL-2 binding domain on the human IL-2R, has been administered IV daily at 5 mg (Group I, n = 9) or 10 mg (Group II, n = 18) for 2 weeks after transplantation in association with prednisone and azathioprine [11]. Clinical and biological tolerance were excellent. A rescue treatment with anti-thymocyte globulin (ATG) was introduced if rejection crises occurred. In this first French series, 3 patients in Group I and only 1 patient in Group II experienced reversible rejection episodes during the first 14 post-transplant days, results significantly better as compared to the historical control. In the second series of 30 recipients treated with 10 mg of mAb daily, only 1 patient had a reversible rejection [12]. There were no life threatening infections and all patients are alive; 97 % of grafts sustain a good function (follow-up 30-210 days). The majority of patients (85 %) developed IgG or IgM antibodies with a maximal response at day 24 for both isotypes.

The randomized trial utilizing adjunctive anti-Tac mAb in human renal recipients is underway at the Brigham and Women's Hospital in Boston (Kirkman RL, personal communication). As in Nantes, antibody treatment is well tolerated. There are fewer early rejection crises; anti-Tac delayes the onset of rejection as compared to conventionally treated patients. Clearly, all these early but still limited data suggest that adjunctive use of anti-IL-2R mAb may be safe and very efficient in preventing early rejection in clinical organ transplantation.

IL-2R Targeted Therapy in Rat Recipients of Organ Allografts

Cardiac Allograft Survival in ART-18 Treated Rats: ART-18, a mouse IgG1 antibody precipitating the p55 subunit of the rat IL-2R molecule inhibits in vitro both the binding of IL-2 to the receptor and IL-2 driven T cell proliferation [13]. Thus, ART-18 recognizes an epitope which is identical to or overlapping with the IL-2 binding domain of the receptor. We have used this material in an attempt to combat acute rejection of heterotopic cardiac allografts in a (LEW × BN) F1 to LEW inbred rat strain combination [14]. ART-18 as a sole modality administered IV for 10 consecutive post-transplant days, increased allograft survival in a dose-dependent fashion to ca. 21 days (acute rejection in untreated hosts occurs within 8 days). Therapy initiated 5 days after grafting, the time of major rejection activity, and continued for a total of 5 days, improved graft survival to ca. 18 days, whereas treatment of two consecutive rejection episodes (5-9 and 15-19 days) with ART-18 extended the survival to ca. 1 month. The outcome of IL-2R targeted therapy was not unique to one strain combination; comparable cardiac graft prolongation was observed in several strongly histoincompatible recipients [15]. To confirm that these results were related to the specificity of ART-18 for IL-2R, a group of animals was treated with ART-62 (IgG1), an antibody which recognizes rat Class 1 MHC antigens and inhibits IL-2 driven T cell growth but does not bind to rat IL-2R [16]. ART-62 applied in the same dose regimen and time course as ART-18, did not influence the tempo of rejection at all [17]. Thus, therapy with ART-18 targeted selectively at IL-2R+ cells can be successfully utilized to delay or treat acute rejection of cardiac allografts in rats.

The Effect of ART-18 Treatment on Lymphocyte Populations Mediating Allograft Rejection: Specifically sensitized CD4+ (T helper) but not CD8+ (T cytotoxic/suppressor) cells, restore allograft rejection in ca. 12 days following transfer into profoundly T cell deficient and otherwise immunologically inert B rat recipients of cardiac allografts; combining both subsets produces a similar effect [18]. A 10-day course of ART-18 started 1 day after cell transfer dramatically prolonged graft survival to ca. 35 days and 19 days in B hosts

repopulated with either Th or both T cell subsets, respectively [17]. Classic acute rejection within 8 days is restored in B recipients following transfer of both T lymphocyte subpopulations supplemented with a course of exogenous IL-2 [18]. However, concurrent administration of ART-18 greatly diminished this response and produced a biological effect as if ART-18 alone, but not IL-2 had been given (graft survival ca. 20 days) [17]. Thus, there is a divergent efficacy of ART-18 in destroying IL-2R bearing cells; the in vivo binding of ART-18 to the targets is not prevented by the presence of IL-2.

Graft Infiltration: Although phenotypic markers are important in identification of cell subpopulations, activation markers are more relevant to actual cell function. The presence and distribution of IL-2R bearing cells have been studied using a panel of mAbs and immunoperoxidase techniques [19]. In untreated LEW recipients, IL-2R+ mononuclear cells were identified within rejecting (LEW × BN) F1 grafts from day 2 after transplantation, with the numbers peaking an days 4-6, when 15-20 % of infiltrating cells bearing leukocyte common antigen (OX1) were also IL-2R+. Interestingly, double-labelling studies revealed that both T cells and macrophages expressed IL-2R. Thus, at day 6 only about half of IL-2R+ cells were positive for a pan-T cell marker (W3/13); the remainder expressed macrophage specific antigen (ED-2). In contrast, rats treated with ART-18 lacked detectable IL-2R+ cells during the first two weeks after transplantation and showed significantly less overall cellular infiltration. However, just prior to ultimate rejection at 21 days, infiltration with IL-2R+ cells increased sharply. Thus, the discrete population of graft infiltrating IL-2R+ T cells and macrophages play a critical role in acute allograft rejection.

The Effect of ART-18 Treatment on Host Alloreactivity: Splenic T cells harvested from engrafted hosts treated with a 10 day course of ART-18 and adoptively transferred into normal, syngeneic secondary recipients significantly improved (LEW × BN) F1 but not third-party (WF) test cardiac allograft survival (ca. 16 days and ca. 8 days, respectively); this suggested a sparing effect of ART-18 upon donor specific T suppressor (Ts) cell activity in vivo [14]. When T cells were fractionated into highly purified negatively selected components, the CD8+ (Tc/s) subset conferred suppression to naive rats following adoptive transfer; in contrast CD4+ (Th) cells were ineffectual [17]. Thus, a population of Ts confined to CD8 phenotype is preserved in cardiac allografted and ART-18 treated rats. A possible explanation of this unexpected phenomenon may be that in vivo CD8+ Ts may lack an IL-2R epitope recognized by ART-18. Alternatively, IL-2R may be expressed dysynchronously upon CD8+ cells. As a consequence, ART-18 therapy may preferentially destroy Th and T cytotoxic lymphocytes (CTL), but not Ts clones.

Indeed, alloagressiveness of CD4+ cells is profoundly depressed in ART-18 treated hosts. Thus, treatment with anti-IL-2R mAb abrogated the capacity of the CD4+ subset to recreate acute rejection following transfer into otherwise immunologically anergic B rats [17]. These cells promoted a late and attenuated rejection at a tempo comparable to that of CD4+ cells from normal untreated rats (ca. 40 days). Moreover, endogenous elaboration of IL-2 by spleen cells was profoundly diminished whereas production of IL-3 was hightened in recipients just completing ART-18 regimen. Thus, ART-18 produces a selective immune defect in the host by sparing CD8+ Ts but depressing CD4+ Th activities. Decreased elaboration of IL-2 concomitantly augments the release of IL-3, a lymphokine which might play a role as Ts growth, activation or maturation factor [20].

As already mentioned, ART-18 treatment halts temporarily the rejection process in grafted rats. However, in contrast to the effect produced by short term Cyclosporine (CsA) treatment [21], the permanent acceptance of cardiac allografts was never observed. Thus, the question arises as to how to increase the therapeutic index of anti-IL-2R mAb therapy. We will discuss this next pointing out the role of antibody isotype, the significance of cellular epitope(s) targeting patterns, and finally putative in vivo synergistic interactions between anti-IL-2R modalities and other immunosuppressive drugs.

IL-2R Targeted Therapy – The Role of Antibody Isotype: Although ART-18 blocks IL-2R in vitro [13], extremely high amounts of mAb would be required to inhibit IL-2 binding in vivo due to the high affinity of IL-2 to the receptor (kD = 10^{-11} M), compared to that of ART-18 (kD = 10^{-9} M) [22]. Obviously, the in vivo beneficial effect has been achieved by the use of mAb in amounts smaller than those required for in vitro inhibition of IL-2 dependent cell growth. Therefore, elimination by (a) complement dependent lysis, (b) antibody-dependent cell cytotoxicity (ADCC), or (c) opsonization and phagocytosis represents the most probable mechanism(s) of action of ART-18 in vivo (Figure). These activities depend on the ability of mAb to bind isotype specific Fc receptors on the respective effector cells. Thus, the isotype of mAb therapeutically employed may be of importance.

To address this issue we produced ART-18 switch variants of the same binding parameters but distinct isotype specificities (IgG2a and IgG2b) as compared to the parental IgG1 clone, and probed their immunosuppressive efficacy in rat cardiac allograft recipients [23]. Indeed, acute rejection could be prevented in a dose dependent fashion following a 10 day treatment with all three ART-18 materials. In contrast, the long-term therapeutic effects depend on

the isotype of antibody employed: IgG2b preparation facilitated the longest graft survival (ca. 28 days), followed by IgG1 (ca. 21 days) and IgG2a, which was least effective (17 days). The survival rate was not additively increased when mAbs of distinct isotypes were administered concomitantly or alternately [24]. Preliminary studies suggest that differences in phagocytosis and in the ability to induce ADCC are primarily responsible for the divergent in vivo efficacy of ART-18 switch variants. Alternatively, treatment of mice with M7/20, an anti-IL-2R mAb of IgM isotype and binding parameters identical to ART-18, obviated delayed hypersensitivity responses in normal but not in complement (C5) deficient mice [25], suggesting that mAbs ideally should fix terminal complement components and inhibit T cell function. As none of our ART-18 preparations bind complement, experiments are underway to generate switch variants which will initiate complement-dependent lysis.

IL-2R Targeted Therapy. The Role of Epitope: ART-65 and OX-39, two new mouse non-cross reacting IgG1 antibodies, recognize distinct epitopes of the rat p55 IL-2R molecule [26, 27]. In contrast to ART-18, neither of these mAbs affects IL-2 drivenT cell growth; OX-39 inhibits binding of IL-2 to its receptor, whereas ART-65 does not. Interestingly, ART-65 given to cardiac allograft recipients in a dose and time course similar to ART-18, did abrogate acute rejection and extended graft survival to ca. 16 days [24]. This observation suggests that (1) anti-IL-2R mAbs do not inhibit expansion of the respective IL-2R+ clones in vivo; (2) inhibition of T cell function is not required for sucessful IL-2R targeted therapy.

The most striking observation came from the studies in which rat recipients of cardiac allografts were treated with ART-18 and ART-65 in combination to target distinct IL-2R epitopes. Concomitant administration of these mAbs to recipient animals in relatively low doses proved highly effective, with 30 % of transplants surviving indefinitely and 50 % undergoing late rejection at ca. 50 days [28]. These results provide evidence that anti-IL-2R mAbs should target simultaneously functionally different epitopes of the IL-2R molecule to obviate rejection effectively. Sparing of phenotypically distinct Ts may contribute to the synergy between ART-18 and ART-65 in vivo. As stated before, CD8+ Ts are spared following ART-18 therapy; in contrast in ART-65 treated hosts it was primarily CD4+ cells which conferred profound specific suppression to naive rats following adoptive transfer and prolonged test graft survival to unprecedented length (ca. 45 days). Whether the long lasting therapeutic effect of the cocktail of mAbs was achieved by preventing association of p55 beta and p75 alpha chains so that no high affinity receptors can be formed, remains to be determined.

In contrast, OX-39 therapy never influenced the tempo of rejection [28, 29]. Apparently, OX-39 interacts with low affinity IL-2 binding and may define the epitope which is outside the cluster recognized by ART-18. Although each mAb interacts specifically with a given epitope in vivo, the three-dimensional structure of the epitope may occur on both related and unrelated molecules [30]. Therefore, OX-39, which in contrast to ART-18 and ART-65 reacts with rat thymic dendritic cells and medullary thymocytes [31], may be actively «captured» by unrelated cells and tissues expressing the common epitope in vivo, and therefore is unable to reach the related targets.

In vivo Synergy Between Anti-IL-2R mAbs and CsA: A series of immunoperoxidase studies revealed that Ia+ MHC Class II antigen expressing cells and activated macropgahes, as determined by OX-3 and A1-3 mAbs, respectively, were abundant in well-functioning cardiac allografts in ART-18 treated hosts; almost no such cells were detected in CsA treated hosts [19]. This observation prompted our efforts to improve the therapeutic index of anti-IL-2R mAbs and to test the adjunctive effect of low dose CsA. Indeed, a combination of ART-18 treatment (which prolongs cardiac allograft survival to ca. 21 days on its own) with subtherapeutic dose of CsA (one-tenth of the effective dose, which is ineffectual by itself) was strikingly synergistic in preventing rejection: one-third of the transplants survived indefinitely with the remainder rejecting at ca. 50 days following a 10 day post-operative therapy [32]. This combination therapy was also very effective in reversing well established rejection, with 20 % of hearts surviving 100 days. Having achieved such biological effect, the results of immunohistological studies were not surprising: no activated macrophages could be found in the cardiac allografts of ART-18 treated recipients concomitantly receiving low dose CsA; similarly, Class II antigen expression was low and indistinguishable from isografts or from allografts from hosts treated with full dose CsA, at both early and late stages after transplantation [33].

These studies demonstrate that subtherapeutic doses of CsA, which by themselves have no discernible immunosuppressive effects, act synergistically with IL-2R targeted therapies. Adjunctive low dose CsA contributes to the development of transplantation unresponsiveness in the induction phase of graft survival presumably by preventing activation and proliferation of alloreactive lymphocytes and macrophages.

These observations of potential major clinical importance prompted trials in other rat experimental models to test putative synergy between anti-IL-2R mAbs and CsA. Although ART-18 treatment with or without adjunctive subtherapeutic CsA therapy abrogated acute rejection of pancreatic islets with 30 % of the transplants accepted permanently, only combination treatment produced normal glucose

levels and normal glucose tolerance in long survivors [34]. Similarly, a short-term treatment with ART-18 in combination with low dose CsA cured the majority of spontaneously developing diabetes BB rats; ART-18 alone halted temporarily the destruction of beta cells by autoreactive lymphocytes and delayed the onset of fatal hyperglycemia but failed to cure BB rats [35]. Modifying the behaviour of small bowel allografts was more difficult. Treatment of small bowel allograft recipients with both modalities doubled graft survival, as compared to ART-18 alone, but did not prevent fatal systemic graft-versus-hot (GvH) disease [36]. Apparently, the antigenic stimulus associated with the large amount of lymphoid tissue in the gut is immunologically overwhelming for the recipient. In contrast, in the local GvH reaction, as determined by a popliteal lymph node assay, ART-18 or ART-65 treatment reduced the GvH index; addition of subtherapeutic doses of CsA completely abolished this classic experimental autoimmune disorder [37]. The most recent results on the use of anti-IL-2R mAbs in conjunction with low dose CsA following transplantation of kidney and skin in both rats [33] and monkeys [38] also validate the concept of striking synergy between both modalities. Inhibition of alloreactivity by influencing different steps in the immune cascade with simultaneous preservation of Ts may explain the reasons of this potentially clinically applicable and warranting further studies phenomenon.

IL-2R Targeted Therapy in Sensitized Hosts: As the majority of data on the use of anti-IL-2R mAbs has accrued in otherwise untreated hosts, parallel studies have been initiated to test the effect of these entities in presensitized animals. We have developed an experimental model in which (LEW × BN) F1 cardiac allografts are uniformly rejected in an accelerated fashion within 48 hr by LEW rats challenged with BN skin grafts 7 or 10 days prior to heart transplantation [24]. This fulminant host response combines both humoral and cellular immune components [39]. Thus, antibody titers in the serum rise to relatively high levels during the rejection episode, whereas thrombosis, hemorrhage and areas of myonecrosis are prominent at the graft site; additionally, an important proportion of the infiltrating cell populations carry IL-2R. This latter observation and the well known presence of IL-2R upon some mitogen/antigen activated B cells in vitro [40] validated the concept of modulating accelerated graft loss by using anti-IL-2R mAbs. ART-18 applied in the same dose regimen and time course as in unsensitized hosts, abrogated the 48 hr accelerated rejection and extended graft survival to ca. 16 days [39]. Addition of subtherapeutic CsA resulted in a modest further graft prolongation (ca. 20 days), whereas combining low doses of ART-18 and ART-65 proved again to be the most effective regimen (graft survival ca. 24 days) [24]. Interestingly, histological evaluation revealed a switch in graft injury from predominantly humoral in sensitized recipients to late pure cellular rejection in the treated hosts. Thus, anti-IL-2R mAbs may be useful clinically in sensitized graft recipients, an observation of potential importance for the development of immunosuppressive means to treat hyperacute/accelerated graft loss.

Concluding Remarks

Immunosuppresive therapy has evolved dramatically from the days of total body x-irradiation and thoracic duct drainage, desperate attempts to save the transplanted organ by preventing its rejection. This cascade of reactions of the host immune system called into play in response to the stimulus of foreign tissue was and still is, two decades later, difficult to control. It is not easy to «cheat nature» by modifying the interlocked gears of the complex machinery of the immune system programmed throughout millions years of evolution. However, as transplants save lives, it is the task of the transplant biologist to devise means of producing host acceptance of the graft without deletorious side effects. This goal remains elusive despite such milestones as the introduction of azathioprine, cyclosporine and monoclonal antibodies.

The purpose of this overview is to show that new and selective immunosuppressive technique using anti-IL-2R mAbs may soon become an important part of recipient treatment. However, several problems still remain and various areas of research require detailed analysis and further clarification. Potential host sensitization to repeatedly administered xenogeneic proteins represents a major drawback, as anti-Ig formation may thwart the prospects of long-term treatment. It is encouraging to report that in preliminary studies, the use of classical deaggregation strategy has allowed us to prevent anti-Ig formation in ART-18 treated rats. Antibody isotype and cellular targeting patterns are critical for the efficient killing of target cells; the concomitant use of mAbs recognizing distinct epitopes of the IL-2R molecule may prove highly beneficial. In future trials, the adjunctive use of anti-IL-2R agents in combination with other immunosuppressive modalities warrants further investigation. Finally, the most recent in vitro and in vivo studies demonstrate that a genetically engineered hybrid of the IL-2 molecule spliced to the diphtheria toxin molecule, which is truncated of its receptor for cell binding, can be used to deliver a lethal hit to high affinity IL-2R bearing T cells. This approach should augment cytodestruction of activated lymphocytes.

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