Title

Anti-interleukin 2 receptor monoclonal antibodies spare phenotypically distinct T suppressor cells in vivo and exert synergistic biological effects.

Journal

Journal of Experimental Medicine, 167(6)

ISSN

0022-1007

Authors

Di Stefano, R
Mouzaki, A
Araneda, D
et al.

Publication Date

1988-06-01

DOI

10.1084/jem.167.6.1981

Brief Definitive Report

ANTI-INTERLEUKIN 2 RECEPTOR MONOCLONAL ANTIBODIES SPARE PHENOTYPICALLY DISTINCT T SUPPRESSOR CELLS IN VIVO AND EXERT SYNERGISTIC BIOLOGICAL EFFECTS

By ROSSELLA DI STEFANO, ATHANASIA MOUZAKI,* DORIAN ARANEDA, TIBOR DIAMANTSTEIN,* NICHOLAS L. TILNEY, and JERZY W. KUPIEC-WEGLINSKI

*From the Surgical Research Laboratories, Harvard Medical School, Department of Surgery, Brigham and Women's Hospital, Boston, Massachusetts; and the *Immunology Research Unit, Klinikum Steglitz, Free University, Berlin, Federal Republic of Germany*

Antigen- or mitogen-induced *de novo* expression of high-affinity IL-2-Rs is a requisite step in the course of T cell activation (1). The effectiveness of selective immunosuppressive therapy in organ transplantation using anti-IL-2-R mAbs has been shown both in experimental settings and by encouraging early results from clinical trials (2, 3). In our studies, ART-18, a mouse anti-rat IgG1 antibody, prolonged the survival of cardiac grafts transplanted across MHC barriers in several inbred rat strain combinations (4, 5). We now demonstrate that anti-IL-2-R mAbs selectively spare in vivo phenotypically distinct T cells with suppressor functions (Ts), and that combined treatment with mAbs against functionally different epitopes may exert strikingly synergistic effects.

Materials and Methods

mAbs. The panel of antibodies included ART-18, ART-65, and OX-39. Their production and initial characterization have been described (6–8). Prepared from mouse ascites, they immunoprecipitate the p55 chain of the rat IL-2-R (9) and contain ~10 mg/ml IgG1 (ART-18 and ART-65) and ~2 mg/ml IgG1 (OX-39). In contrast to ART-18, neither of these mAbs affects IL-2-driven T cell proliferation; OX-39 and ART-18 inhibit binding of IL-2 to its receptor, whereas ART-65 does not (6–8). Mouse-clarified ascites (IgG1, ~5 mg/ml) produced by MOPC 21 tumor line (Bionetics Laboratory Products, Kensington, MD) was used as a control.

mAb Binding Assay. Il-2-dependent rat G2 T cells (10^6) were incubated for 1 h at 4°C with increasing concentrations of ^{125}I-labeled ART-18, ART-65, or OX-39 (10). The cells were then washed, separated from the unbound labeled material, and counted in a gamma counter. The number of binding sites and the affinity of each mAb were calculated by Scatchard plot analysis.

Animals and Grafting Technique. Inbred male adult Lewis rats (LEW, $RT1^l$) served as recipients of cardiac allografts from (LEW × BN)F_1 ($RT1^{l/n}$) hybrid or Wistar Furth (WF, $RT1^u$) donors (Harlan Sprague Dawley, Inc., Indianapolis, IN). Hearts were transplanted

This work was supported by Grant-In-Aid 861424 from the American Heart Association and National Institutes of Health grants 1R01 AI-23847 and 5R01 AI-19071-13. R. Di Stefano was supported by a fellowship from Sigma-Tau. Address correspondence to J. W. Kupiec-Weglinski, Surgical Research Laboratory, Harvard Medical School, 25 Shattuck St., Boston, MA 02115.

to recipient abdominal great vessels using microvascular techniques. Graft function was assessed daily by palpation through the host flank; rejection was taken as the time of complete cessation of myocardial contractions.

mAb Treatment. ART-18, ART-65, and OX-39 mAbs were administered to experimental animals intravenously at doses of 25–300 μg/kg per d for 10 d starting the day of transplantation. There were 5–9 animals in each experimental group.

Cell Preparations. Single spleen cell (SL) suspensions were prepared by mincing and expressing SL through 60-gauge stainless steel mesh into RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with 5 mM Hepes, 2 mM L-glutamine, and 10% heat-inactivated FCS (Associated Biomedic System, Buffalo, NY). Erythrocytes were lysed by brief treatment with 0.83% Tris-buffered ammonium chloride (pH 7.21). Nonadherent T cell-enriched (>95%) populations from nylon wool columns were fractionated into $CD4^+$ (T helper/inducer, $W3/25^+$) and $CD8^+$ (T suppressor/cytotoxic, $OX8^+$) subsets, as described (11). Only nonadherent negatively selected T cells (>90% viable, enriched >95% for the desired subpopulation) were adoptively transferred into syngeneic normal rats that received a test graft 24 h later. There were 4–7 animals in each group.

Statistical Analysis. The statistical differences between experimental groups were ascertained using Student's t test.

Results

Binding Parameters for Anti-IL-2-R mAbs. Scatchard analysis revealed distinct binding of iodinated mAbs to G2 target cells. The highest affinity was for OX-39 (k_d = 8.26 × 10^{-10}), lower for ART-65 (k_d = 1.2 × 10^{-9}), and least for ART-18 (k_d = 1.91 × 10^{-9}). In contrast, ART-18 had the maximal number of molecules bound per G2 cell (75,000), followed by OX-39 (61,185), and ART-65 (27,702).

Therapeutic Efficacy of Anti-IL-2-R mAbs. ART-18 therapy (300 μg/kg per d for 10 d, i.v.) prolongs the survival of (LEW × BN)F_1 cardiac grafts in LEW recipients to ~21 d (acute rejection in untreated hosts occurs within 8 d, $p < 0.001$) (4). Similarly, a 10-d course of ART-65 (300 μg/kg per d) extended graft survival (MST ± SD = 15.5 ± 4.4 d, $p < 0.01$, as compared with ART-18-modified hosts). In contrast, therapy with OX-39 or with the control IgG1 antibody never influenced the tempo of rejection (8 ± 1 and 7 ± 1 d, respectively).

The Selective Effect of Anti-IL-2-R mAbs on T Cell Subsets. Recent work from this laboratory has demonstrated sparing of Ts by ART-18 treatment (4, 12). Donor-specific test graft survival is prolonged in naive syngeneic rats to 15.0 ± 1 and 15.5 ± 0.9 d after adoptive transfer of 100 × 10^6 unseparated SL or 50 × 10^6 $CD8^+$ T cells harvested from mAb-conditioned animals 10 d after transplantation, respectively ($p < 0.001$); $CD4^+$ cells (40–50 × 10^6) were ineffectual (8.5 ± 0.5 d). In the present studies, transfer of 100 × 10^6 SL from ART-65-treated LEW recipients bearing well-functioning (LEW × BN)F_1 transplants extended the survival of specific test cardiac allografts to 13.6 ± 2.9 d ($p < 0.001$, as compared with untreated controls). However, when SL from ART-65-treated recipients were separated into highly purified and non-overlapping component parts, the $CD4^+$ subset (50 × 10^6 cells) conferred profound suppression to normal LEW, with prolongation of (LEW × BN)F_1 test grafts to lengths (MST ± SD = 45 ± 17.5 d) unprecedented in our previous experience; transfer of the same number of $CD8^+$ cells was less effective (14.5 ± 0.7 d, $p < 0.001$). The third-party (WF) test grafts were always rejected within 10 d in the comparable groups of secondary recipients. All the above experiments subsequently were repeated 4–7 times; the results were uniformly the same. Thus, IL-2-

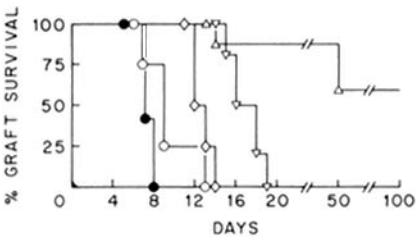

FIGURE 1. (LEW × BN)F_1 cardiac allograft survival in LEW recipients treated with ART-18 and/or ART-65 anti-IL-2-R mAb(s) (25 μg/kg per d, i.v.; there were 5–9 animals in each experimental group). (○) ART-65 (treatment for 10 consecutive post-transplant days); (◇) ART-18 (treatment for 10 consecutive post-transplant days); (▽) ART-18 (treatment for the first 5 post-transplant days) + ART-65 (treatment for the second 5 post-transplant days); (△) ART-18 + ART-65 (treatment for 10 consecutive post-transplant days; (●) untreated controls.

R-targeted therapy selectively spares phenotypically distinct populations of Ts: $CD8^+$ in ART-18-treated animals and primarily $CD4^+$ in ART-65-treated hosts.

The Effect of Combined ART-18 Plus ART-65 mAb Treatment. Rat recipients of cardiac allografts were treated with a combination of ART-18 and ART-65 to target distinct epitopes of the IL-2-R molecule, as well as to influence the selective in vivo effects of mAbs upon host Ts. Concomitant administration of ART-18 and ART-65 for 10 d after transplantation in relatively low doses (25 μg/kg per d), only marginally operative on their own (graft survival = 13.0 ± 1 and 9.5 ± 2.5 d, respectively), proved highly effective, with 3 of 10 cardiac allografts surviving indefinitely, five undergoing rejection at periods >50 d, and the remaining grafts surviving ~3 wk (Fig. 1). Treatment with ART-18 for the first 5 d after transplantation followed by ART-65 therapy for the subsequent 5 d was less striking (16.8 ± 1.6 d). Thus, ART-18 and ART-65 in combination exert an important synergistic effect in vivo by simultaneously targeting IL-2-R^+ cells activated in response to the allogeneic graft. In contrast, therapy combining OX-39 with ART-18 did not improve survival beyond that observed after ART-18 antibody alone (data not shown).

Discussion

The present studies were designed to compare and correlate the in vitro and in vivo immunosuppressive properties of ART-18, ART-65, and OX-39 (Table I), noncrossreactive IgG1 antibodies that recognize distinct epitopes on the p55 β chain of the rat Il-2-R (9). Several questions can be posed based on the results of the experiments.

Why Are mAbs of the Same Isotype and Common Specificity for the p55 Subunit of the IL-2-R Molecule not Equally Effective In Vivo? These experiments stress the lack of correlation between the in vitro binding parameters of anti-IL-2-R mAbs and their biological efficacy in vivo. ART-18, despite its lower apparent affinity for the receptor, exhibits potent immunosuppressive properties in vivo. In contrast, OX-39 in the same dose did not influence acute rejection of cardiac allografts despite demonstrating the highest affinity for its epitope and covering large numbers of binding sites per target cell in vitro. 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 (13). For example, ART-62, a mouse anti-rat IgG1 antibody that blocks T cell function and inhibits Il-2-dependent T cell proliferation in vitro, lacks biological activity in vivo, despite recognizing broadly the epitope of the rat MHC Class I antigen present on various cells including erythrocytes (12). It is

TABLE I

The In Vitro and In Vivo Properties of Mouse Anti-rat IL-2-R mAbs

		In vitro activities*				In vivo activities		
		Binding parameters		Inhibition of:			Ts sparing§	
mAb	Isotype	Affinity kD	Number of binding sites	IL-2 binding	IL-2 proliferation	Graft survival‡	$CD8^+$	$CD4^+$
ART-18	IgG1	1.91×10^{-9}	75,000	+	+	21 d	+	–
ART-65	IgG1	1.20×10^{-9}	27,702	–	–	16 d	+	+ + +
OX-39	IgG1	8.26×10^{-10}	61,185	+	–	8 d	ND	ND

* The in vitro properties of mAbs were tested using the IL-2-dependent rat G2 T cell line. The apparent affinities of mAbs and the number of binding sites on target cells were calculated using Scatchard plot analysis. The effects of mAbs upon IL-2 binding and IL-2-dependent T cell proliferation were tested as described (6-8).

‡ The therapeutic efficacy of mAbs was evaluated in the rat cardiac allograft model ([LEW × BN]F_1 → LEW; acute rejection in untreated recipients occurs within 8 d). Antibodies were administered for 10 consecutive days after transplantation (300 μg/kg per d i.v.). Mean graft survival times are shown (n = 5-9).

§ The "sparing" of putative Ts was tested in adoptive transfer studies. Negatively selected $CD8^+$ ($OX8^+$ $W3/25^-$), or $CD4^+$ ($W3/25^+$ $OX8^-$) spleen T cells ($40-50 \times 10^6$) from ART-18 or ART-65 mAb-treated heart graft recipients were administered into naive syngeneic rats that were challenged with a donor-specific test cardiac allograft 24 h later. Mean test graft survival times (n = 4-7): 14-16 d (+); 45 d (+ + +); 8-10 d (–).

possible that OX-39 may be actively "captured" by unrelated cells and tissues expressing the common epitope in vivo, and therefore, it is unable to reach the related targets. Indeed, recent studies demonstrate that this mAb, in contrast to ART-18, reacts with ~3% of rat thymic dendritic cells and ~2% of rat thymocytes (8).

Is Blocking of T Cell Activity by Anti-IL-2-R mAbs Required for Their In Vivo Effects? The present results in an allograft model complement recently reported local graft-vs.-host reaction trials that demonstrate the immunosuppressive efficacy of ART-65, an antibody that in contrast to ART-18 affects neither IL-2 binding nor IL-2-dependent T cell growth (7). Inhibition of T cell function may not be a prerequisite for successful IL-2-R-targeted therapy as the in vivo mode of action of these mAbs is to eliminate, rather than to inhibit expansion of, IL-2-R^+ effector cells. Preliminary data on the use of ART-18 isotype switch variants suggest antibody-dependent cell cytotoxicity to be primarily responsible for the effect observed in our studies (14). Alternatively, treatment with M7/20, a rat anti-mouse IL-2-R mAb of IgM isotype and binding parameters identical to ART-18, obviated delayed-type hypersensitivity responses in normal but not in complement deficient mice (15), suggesting that mAbs ideally should fix terminal complement components and inhibit T cell function.

Why Do Anti-IL-2-R mAbs Selectively Spare Phenotypically Distinct T Cell Subsets? The heavy glycosylation of the β chain of the IL-2-R molecule may explain the discrepancy between the molecular mass of the receptor calculated from the amino acid sequence (30 kD) and the actual molecular mass of the isolated receptor (55 kD) (16).

Non-random glycosylation of the IL-2-R molecule on host lymphocytes may lead to divergent accessibility of T cell subsets by anti-IL-2-R mAbs with resultant sparing of distinct Ts subpopulations. Thus, ART-18 treatment does not affect the majority of $CD8^+$ Ts but inactivates the $CD4^+$ subset including $CD4^+$ Ts, whereas ART-65 therapy preserves primarily $CD4^+$ Ts. The practical relevance of this observation is of major importance, as sparing of phenotypically distinct Ts may contribute to

the unprecedented graft prolongation in rats treated with the combination of ART-18 and ART-65. It is also conceivable that ART-18 and ART-65 bind to the epitopes on different β chains of $CD4^+$ and $CD8^+$ cells; alternative mRNA splicing may occur (17). It remains to be determined whether the long-lasting therapeutic effect after ART-18 plus ART-65 treatment was achieved by preventing association of p55 β and p75 α chains, so that no high-affinity receptors can be formed.

Summary

The therapeutic efficacies of ART-18, ART-65, and OX-39, mouse antibodies of IgG1 isotype recognizing distinct epitopes of the p55 β chain of the rat IL-2-R molecule, were probed in LEW rat recipients of (LEW × BN)F_1 heterotopic cardiac allografts (acute rejection in untreated hosts occurs within 8 d). A 10-d course with ART-18 prolongs graft survival to ~21 d ($p < 0.001$). Therapy with ART-65, but not with OX-39, was effective (graft survival ~16 and 8 d, respectively). Anti-IL-2-R mAb treatment selectively spared T cells with donor-specific suppressor functions; the $CD8^+$ ($OX8^+$ $W3/25^-$) fraction from ART-18-modified recipients, and primarily the $CD4^+$ ($W3/25^+$ $OX8^-$) subset from ART-65-treated hosts conferred unresponsiveness to naive syngeneic rats after adoptive transfer, increasing test graft survival to ~16 and 45 d, respectively. Concomitant administration of ART-18 and ART-65 to recipient animals in relatively low doses exerted a strikingly synergistic effect, with 30% of the transplants surviving indefinitely and 50% undergoing late rejection over 50 d. These studies provide evidence that anti-IL-2-R mAbs selectively spare phenotypically distinct T cells with suppressor functions. The data also suggest that in vivo targeting of functionally different IL-2-R epitopes may produce synergistic biological effects.

We thank Dr. A. F. Williams and Dr. M. J. Dallman for supplying us with OX-39 mAb, Dr. H. Wonegeit for providing G2 cell line, and Dr. C. B. Carpenter and Dr. T. B. Strom for helpful discussion.

Received for publication 28 December 1987 and in revised form 28 March 1988.

References

1. Smith, K. A. 1984. Interleukin 2. *Annu. Rev. Immunol.* 2:319.
2. Diamantstein, T., H. Osawa, R. L. Kirkman, M. E. Shapiro, T. B. Strom, N. L. Tilney, and J. W. Kupiec-Weglinski. 1987. Interleukin 2 receptor-A target for immunosuppressive therapy. *In* Transplantation Reviews, Vol. 1. P. J. Morris and N. L. Tilney, editors. Grune & Stratton, New York. 177–196.
3. Soulillou, J. P., P. Peyronnet, B. Le Mauff, M. Hourmant, D. Olive, C. Mawas, M. Delaage, M. Hirn, and Y. Jacques. 1987. A monoclonal antibody directed against interleukin 2 receptor prevents rejection of human kidney grafts. *Lancet.* 1:1339.
4. Kupiec-Weglinski, J. W., T. Diamantstein, N. L. Tilney, and T. B. Strom. 1986. Therapy with monoclonal antibody to interleukin 2 receptor spares suppressor T cells and prevents or reverses acute allograft rejection in rats. *Proc. Natl. Acad. Sci. USA.* 83:2624.
5. Kupiec-Weglinski, J. W., W. Padberg, L. C. Uhteg, E. Towpik, R. H. Lord, L. Ma, T. Diamantstein, T. B. Strom, and N. L. Tilney. 1987. Anti-interleukin 2 receptor (IL-2R) antibody against rejection of organ grafts. *Transplant. Proc.* 19:591.
6. Osawa, H., and T. Diamantstein. 1983. The characteristics of a monoclonal antibody

that binds specifically to rat T-lymphoblasts and inhibits IL-2 receptor functions. *J. Immunol.* 130:51.

7. Mouzaki, A., H. D. Volk, H. Osawa, and T. Diamantstein. 1987. Blocking of IL-2 binding to the receptor is not required for the in vivo action of anti-IL-2 receptor mAb. I. The production, characterization and in vivo properties of a new mouse anti-rat IL-2R mAb that reacts with an epitope different to the one that binds to IL2 and the mAb ART18. *Eur. J. Immunol.* 17:335.
8. Paterson, D. J., W. A. Jefferies, J. R. Green, M. R. Brandon, P. Cortese, M. Puklavec, and A. F. Williams. 1988. Antigens of activated rat T lymphocytes including a molecule of 50,000 Mc restricted to CD4 positive T blast. 1987. *Mol. Immunol.* 24:1281.
9. Mouzaki, A., and T. Diamantstein. 1987. Four epitopes on the rat 55 KD subunit of the interleukin 2 receptor as defined by newly developed mouse anti-rat interleukin 2 receptor monoclonal antibodies. *Eur. J. Immunol.* 17:1661.
10. Bolton, A. E., and W. M. Hunter. 1981. The labelling of proteins to high specific radioactivities by conjugation to a 125 I-containing acylating agent. *J. Biochem.* 133:529.
11. Duarte, A. J. S., C. B. Carpenter, and T. B. Strom. 1982. Expression of T cell differentiation antigens and Ia on rat cytotoxic T lymphocytes. *J. Immunol.* 128:580.
12. Kupiec-Weglinski, J. W., W. Padberg, L. C. Uhteg, L. Ma, H. R. Lord, D. Araneda, T. B. Strom, T. Diamantstein, and N. L. Tilney. 1987. Selective immunosuppression with anti-interleukin 2 receptor targeted therapy: helper and suppressor cell activity in rat recipients of cardiac allografts. *Eur. J. Immunol.* 17:313.
13. Kearney, J. F. 1984. Hybridoma and monoclonal antibodies. *In* Fundamental Immunology. W. E. Paul editor. Raven Press, New York. 751–766.
14. Kupiec-Weglinski, J. W., R. Di Stefano, K. G. Stunkel, R. Grutzmann, P. Theisen, D. Araneda, N. L. Tilney, and T. Diamantstein. 1988. Anti-interleukin 2 receptor monoclonal antibody (IL-2R mAb) therapy in rat recipients of cardiac allografts: the role of antibody isotype. *Transplant. Proc.* 20:272.
15. Kelley, V. E., G. N. Gaulton, and T. B. Strom. 1987. Inhibitory effects of delayed-type hypersensitivity: the role of complement and epitope. *J. Immunol.* 138:2771.
16. Shimizu, A., S. Kondo, S. Takeda, J. Yodoi, N. Ishida, H. Sabe, H. Osawa, T. Diamantstein, T. Nikaido, and T. Honjo. 1985. Nukleotide sequence of mouse IL-2 receptor of cDNA and its comparison with the human IL-2 receptor sequence. *Nucleid Acids Res.* 13:1505.
17. Leonard, W. J., J. M. Depper, M. Kanehisa, M. Kronke, N. J. Peffer, P. B. Svetlik, M. Sullivan, and W. C. Greene. 1985. Structure of the human interleukin 2 receptor gene. *Science (Wash. DC).* 230:633.