Transplant Int (1992) 5 [Suppl 1]: S684–S687

TRANSPLANT
International

Rejection prophylaxis with interleukin-2 receptor antibody BT 563: mechanisms of action on human cells

C. Herwartz[1], J. Steinmann[1], U. Bethke[4], R. Engemann[3], J. Gassel[3], S. Hoffmann[2], G. Leimenstoll[2], W. Timmermann[3], and W. Müller-Ruchholtz[1]

[1] Institute for Immunology, [2] Department of Nephrology, Kiel University, Kiel, Germany
[3] Department of Surgery, Würzburg University, Würzburg, Germany
[4] Biotest Pharma, Dreieich, Germany

Abstract. We investigated the in vitro immunosuppressive effect of BT 563, a monoclonal antibody, against the α-chain of the human interleukin-2 (IL-2) receptor (p 55), which has been used to prevent transplant rejection in several clinical trials. We also measured the proliferative T cell alloresponse and pCTL frequencies of BT 563-treated kidney transplant patients. In mixed lymphocyte cultures BT 563 caused a reduction of T cell proliferation to about 50%. This could not be reversed by the addition of exogenous IL-2. A more effective reduction (80%) was seen in the generation of cytotoxic T cells from CML cultures and at the clonal level. The specific T cell response after preincubation with antigen and BT 563 was not reduced so that BT 563 did not induce tolerance. The in vitro findings indicated that BT 563 had a significant but incomplete immunosuppressive effect. This correlated with the clinical course and ex vivo analysis of PBL from BT 563-treated patients after kidney transplantation.

Key words: Human IL-2 receptor – Monoclonal antibody – Graft-rejection

The human interleukin-2 (IL-2) receptor consists of two glycoproteins, p55 and p75, which by themselves display low and intermediate affinity for IL-2 and combine to form a high affinity receptor complex.

BT 563 is an IgG1 mouse monoclonal antibody, developed by Wijdenes [6], which is directed against the p55 chain of the IL-2 receptor. It blocks ligand binding to both low and high affinity receptors. Since only activated T lymphocytes express p55 following organ transplantation, the suppressive effect should concentrate on transplant reactive cells. In several clinical trials BT 563 has been used to prevent rejection after solid organ transplantation and to treat graft-versus-host disease after bone marrow transplantation.

Offprint requests to: Jörg Steinmann, Inst. f. Immunology, Kiel University, Michaelisstr. 5, D-2300 Kiel 1, Germany

Initial experiments have revealed that BT 563 does not deplete target cells and that the blocking of IL-2 binding is noncompetitive. To increase our understanding of the clinically observed immunosuppressive effect, we investigated the mode of action of BT 563 in various T cell assays in vitro. Additionally, anti-donor T cell reactivity in kidney transplant patients was measured before, during and after BT 563 rejection prophylaxis.

Materials and methods

BT 563. BT 563 is a mouse IgG1 monoclonal antibody against the human IL-2 receptor. It is produced and purified by Biotext Pharma according to the guidelines of the European Communities: "On the production and quality control of monoclonal antibodies of murine origin intended for use in man".

Cells. Peripheral blood lymphocytes (PBL) from healthy donors were obtained by density gradient centrifugation of heparinized blood.

MLR. One-way mixed lymphocyte reactions (MLR) were carried out with PBL from different donors. We seeded 5×10^4 responder cells and 5×10^4 irradiated stimulator cells per well into 96-well round-bottomed microculuture plates in 200 µl RPMI 1640, supplemented with 15% AB serum, glutamine and penicillin/streptomycin. After 96 h the microcultures were pulsed with 37 kBq/well ^{3}H-methylthymidine for 24 h and harvested onto glass fiber filters. Tritium incorporation was measured by liquid scintillation spectroscopy.

CML. CML assays were performed in a manner similar to MLR but evaluated for cytotoxicity by ^{51}chromium release on day 7.

Limiting dilution assays. Limiting dilution assays for donor-reactive CTL precursors were performed according to the consensus protocol of the European pCTL Workshop [12]. Briefly, graded numbers of PBL were cocultured with 5×10^4 irradiated kidney donor spleen cells in round-bottomed microtiter plates as described above. We added 10 U/ml recombinant IL-2 in fresh medium on days 3 and 6. On day 12 ^{51}Cr-labelled target cells were added directly to the microcultures. The supernatant was removed after 4 h and the amount of ^{51}Cr released was determined by gamma counting. Microcultures were defined as positive when counts were higher than those of con-

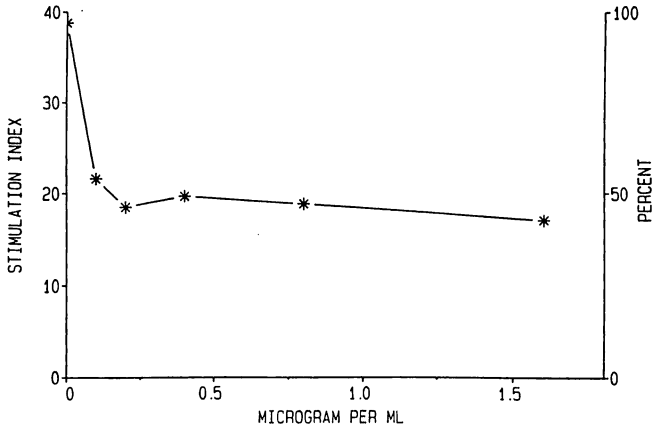

Fig. 1. Reduction of the stimulation index in mixed lymphocyte cultures by increasing concentrations of BT 563

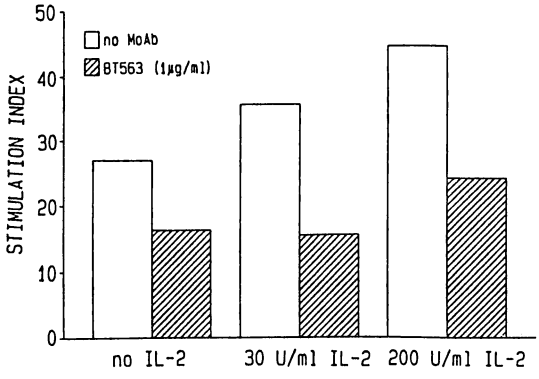

Fig. 2. Influence of exogenous IL-2 on the suppressive effect of BT 563 in mixed lymphocyte cultures

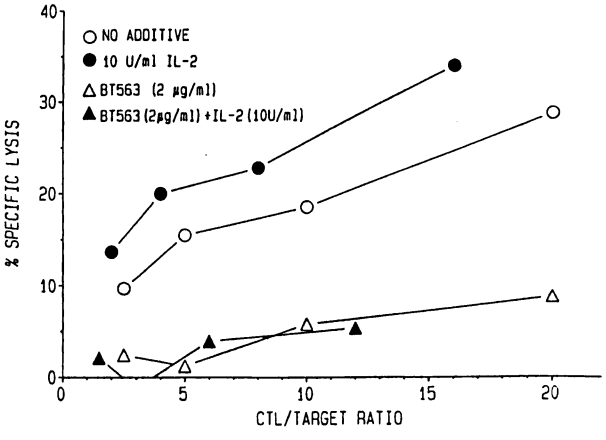

Fig. 3. Inhibition of cell-mediated lysis by BT 563 with and without exogenous IL-2

trols (spontaneous release, irradiated stimulators alone) plus 3-fold standard deviation. Due to the assay setup, pCTL anti-donor frequencies lower than 3 per million were not signfiicantly different than zero. Frequencies were calculated according to Taswell (Lausanne) using the Strijbosch computer program distributed by F. Claas, Eurotransplant, Leiden (Netherlands). By measuring the proliferative response of the sequentially diluted PBL on day 10, expressed as ^{3}H-thymidine uptake, it was possible to calculate the frequency of clonable T-cells.

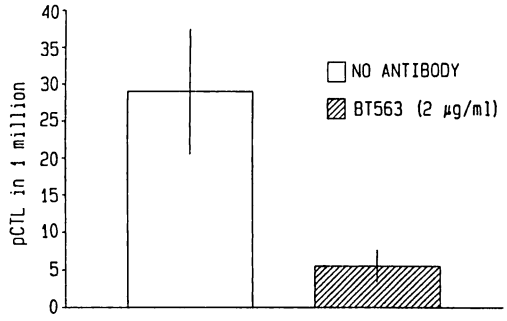

Fig. 4. Reduction of pCTL frequencues by BT 563, measured in limiting dilution cultures

Results and discussion

Effects of BT 563 in vitro

BT 563 inhibited the MLR. The clinically adjusted concentration of BT 563 was 2 µg/ml. In in vitro MLR we tested the effect of the antibody up to 5 µg/ml. As shown in Fig. 1, as little as 0.25 µg/ml BT 563 reduced the stimulation index to about 50%. Higher concentrations did not, however, increase the suppressive effect.

IL-2 did not overcome the immunosuppressive effect. Figure 2 showed that IL-2 enhanced proliferation in MLR, but that the proportional suppressive effect of pretreatment with saturating concentrations of BT 563 was not influenced. This finding indicated that IL-2 and BT 563 do not share the same epitope on the α-chain of the receptor and that the inhibitory effect of BT 563 was not due to competition.

BT 563 reduced the generation of cytotoxic T cells. The IL-2 dependent differentiation of cytotoxic T cells was more effectively influenced by BT 563. Figure 3 demonstrates that 2 µg/ml BT 563 strongly reduced the in vitro development of alloreactive T cells in the standard CML assay. Also this suppressive effect could not be overcome by the addition of exogenous IL-2. This finding could also be demonstrated at the clonal level by performing limiting dilution analysis. As shown in Fig. 4, the frequency of clonable alloreactive cells from the peripheral blood was reduced to 20%. This appeared to indicate that the antibody treatment left out a T cell population which proliferates and differentiates in the presence of BT 563.

BT 563 did not anergize antigen-specific T cells. To determine whether BT 563 was able to induce anergy, we investigated the antigen-specific response of PBL after 1 week of preincubation with antigen and BT 563. Compared to fresh PBL, we could not detect a significant reduction of the pCTL-frequency as shown in Fig. 5.

Concerning the proliferative response, similar results were seen in secondary MLR (data not shown). These findings indicated that BT 563 did not induce tolerance in alloreactive T cells. Taken together, our in vitro data demonstrated that BT 563 had a significant but limited immunosuppressive effect on the alloreactive T cell response. To investigate the clinical effect of BT 563, we analysed

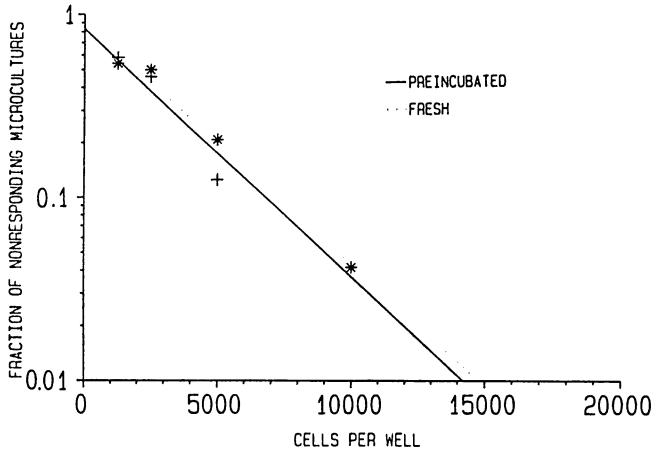

Fig. 5. pCTL frequencies of preincubated PBL compared to fresh cells

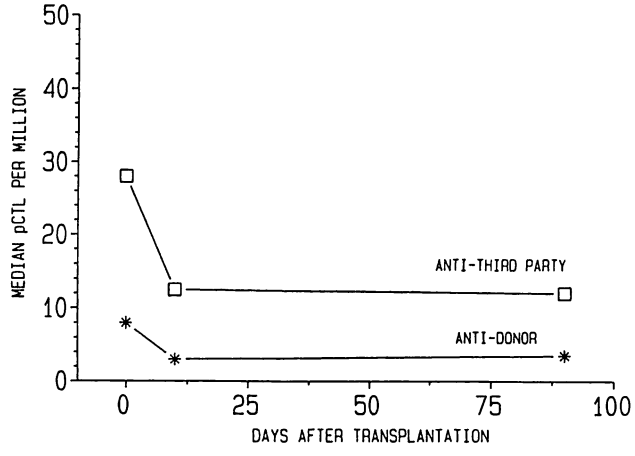

Fig. 7. Mean pCTL frequencies of BT 563-treated kidney transplant patients

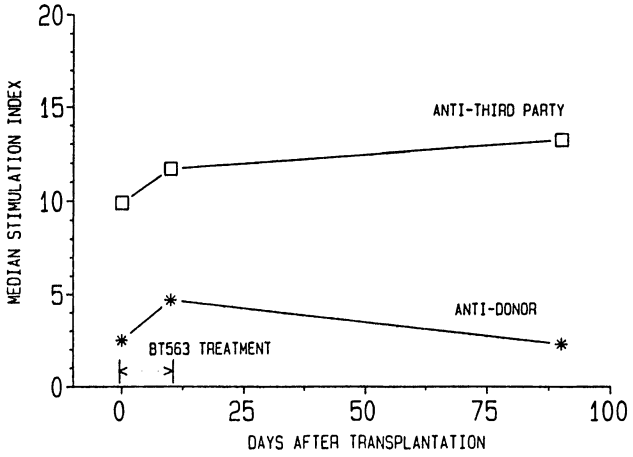

Fig. 6. Mean stimulation index in MLR of nine kidney transplant patients before, during and after BT 563 treatment

PBL from kidney transplant patients before, during and after BT 563 rejection prophylaxis.

Effects of BT 563 in vivo in a phase II clinical trial

Nine kidney transplant patients were treated with BT 563, 10 mg per day, from day 0 until day 9 after transplantation, in addition to cyclosporin A (CsA) and prednisolone. One patient rejected the kidney in association with CMV infection on day 28, four patients had stable graft function after episodes of rejection and four patients showed no signs of rejection.

BT 563 treatment did not reduce MLC-reactivity and pCTL frequency. PBL from the kidney transplant patients were prepared from day 0, 10 and 90 after transplantation and both proliferative activity and pCTL frequencies were measured. The general allogeneic reactivity was determined by coculturing with HLA-mismatched third-party cells and compared to the specific reactivity against HLA-matched donor cells, which is generally lower due to pretransplant typing. As shown in Fig. 6 the mean stimulation index increased slightly between day 0 and day 10. This correlated with an increase in the percentage of IL-2 receptor α-chains expressing T cells and the serum levels of interleukin-6 IL-6 (Data not shown). On day 90 the mean MLR stimulation index was at the pretransplant level again, whereas the anti-third-party index was further but not significantly increased.

In contrast to the MLR stimulation index, the mean pCTL frequency was significantly reduced against donor and third-party on day 10 and remained on this low level until day 90 as shown in Fig. 7. Since Kosugi et al. [8] have demonstrated that CsA abrogates the development of mature T cells, we interpreted this target-independent effect as CsA determined, rather than due to BT 563. However, a controlled clinical study is necessary to clarify this important point.

In summary, in vitro, ex vivo and clinical data in kidney transplantation indicated that BT 563 has an immunosuppressive effect. So far we are not able to support the hypothesis that the antibody induces donor-specific nonreactivity.

References

1. Audrain M, Boeffard F, Soulillou JP, Jacques Y (1991) Synergistic action of monoclonal antibodies directed at p55 and p75 chains of the human IL-2 receptor. J Immunol 146:884
2. Blaise D, Olive D, Hirn M, et al (1991) Prevention of acute GVHD by in vivo use of anti-interleukin-2 receptor monoclonal antibody (33B3.1) a feasibility trial in 15 patients. Bone Marrow Transplant
3. Brown PS, Parenteau GL, Dirbas FM, et al (1991) Anti-Tac-H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival. Proc Natl Acad Sci USA 88:2663
4. Carpenter CB, Kirkman RL, Shapiro ME, et al (1989) Prophylactic use of monoclonal anti-IL-2 receptor antibody in cadaveric kidney transplantation. Am J Kidney Dis 14:54
5. Chabannes D, Mauff BL, Hallet MM, Jacques Y, Soulillou JP (1988) Effect of cyclosporine on interleukin 2 receptor expression in a human alloreactive T cell clone. Transplantation 46:97
6. Hervé P, Wijdenes J, Bergerat JP, Milpied N, Gaud C, Bordigoni P (1988) Treatment of acute graft-versus-host disease with monoclonal antibody to IL-2 receptor. Lancet II:1072

7. Jàcques Y, Audrain M, Boeffard F, Soulillou JP (1991) Concerted action of monoclonal antibodies directed at the p55 and p75 chains of the human interleukin-2 receptor. Transplant Proc 23:1068
8. Kosugi A, Sharrow SO, Shearer GM (1989) Effect of Cyclosporin A on Lymphocytes. I. Absence of mature T cells in thymus and periphery of bone marrow transplanted mice treated with cyclosporin A. J Immunol 142:3026
9. Kupiec-Weglinski JW, Mariani G, Tanaka K et al (1989) Biodistribution of anti interleukin 2 receptor monoclonal antibodies correlates with their therapeutic efficacy following transplantation. Cell Immunol 123:148
10. Plaetinck G, Come MC, Corthesy P et al (1990) Control of IL-2 receptor-α expression by IL-1, tumor necrosis factor and IL-2. J Immunol 145:3340
11. Read MH, Shapiro ME, Strom TB et al (1989) Prolongation of primate renal allograft survival by anti-Tac, an anti-human IL-2 receptor monoclonal antibody. Transplantation 47:55
12. Sharrock CEM, Kaminski E, Man S (1990) Limiting dilution analysis of human T cells: a usefull clinical tool. Immunol Today 11:281
13. Turka LA, Carpenter CB, Yunis EJ, Milford EL (1989) Selective sparing of suppressor cells generated in mixed lymphocyte response by an anti interleukin-2 receptor antibody. Transplantation 47:182
14. Zola H, Weedon H, Thompson GR, Fung MC, Ingley E, Hapel AJ (1991) Expression of IL-2 receptor p55 and p75 chains by human B lymphocytes: effects of activation and differentiation. Immunology 72:167