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T. Van Gelder
C. R. Daane
L. M. B. Vaessen
C. J. Hesse
B. Mochtar
A. H. M. M. Balk
W. Weimar

In vitro and in vivo effects of BT563, an anti-interleukin-2 receptor monoclonal antibody

T. Van Gelder (✉) · C. R. Daane
L. M. B. Vaessen · C. J. Hesse · W. Weimar
Department of Internal Medicine,
University Hospital Rotterdam,
Dr. Molewaterplein 40,
3015 GD Rotterdam, The Netherlands

B. Mochtar · A. H. M. M. Balk
Thorax Center,
University Hospital Rotterdam
Dr. Molewaterplein 40,
3015 GD Rotterdam, The Netherlands

Abstract BT563, a murine anti-IL-2R MoAb, was found to be more potent than anti-Tac in inhibiting proliferation in the mixed lymphocyte reaction. Results obtained with 33B3.1 in these experiments were similar to those with BT563. The anti-IL-2R MoAb 2A3 was shown to be a suitable agent for monitoring the effect of BT563 on peripheral blood. IL-2R-positive cells were not detected in peripheral blood samples from 1 h after the first dose until 8 days after the last dose. Plasma trough levels were measured in patients receiving 5 or 10 mg daily. The administration of BT563 to allograft recipients did not lead to clinically significant side effects.

Key words Interleukin-2 receptor
BT563 · MLR
Rejection prophylaxis

Introduction

Clinical organ transplantation has become a routine medical procedure. Although immunosuppressive therapy has improved considerably, graft rejection and complications of immunosuppression, such as the occurrence of infections and the induction of malignancies, still bother doctor and patient all too frequently. Monoclonal antibodies (MoAbs) directed against the interleukin-2 receptor (IL-2R) are one of the results of the continuing search for better immunosuppressive agents [4]. The anti-IL-2R MoAbs anti-Tac [3] and 33B3.1 [5] are the two best documented examples of this new class of immunosuppressive agents. We describe in vitro and in vivo results with the anti-IL-2R MoAb BT563.

Patients and methods

BT563 (Biotest Laboratories, Dreieich – Germany) is a murine anti-IL-2R MoAb of te IgG1-kappa isotype [1]. Anti-Tac is a murine anti-IL-2R IgG2a MoAb [2] and 2A3 is another murine anti-IL-2R IgG1 MoAb (Becton Dickinson). 33B3.1 is a rat IgG2a MoAb (Immunotech, France). 2A3 was shown to bind the IL-2R regardless of prior binding of BT563 (data shown in results).

In vitro experiments

Peripheral blood lymphocytes (PBL) of healthy blood donors were cultured in a mixed-lymphocyte reaction (MLR) for 7 days in RPMI-1640 (Dutch modification) supplemented with 10% human serum, 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM glutamine at 37 °C. Responder cells (5×10^4) were stimulated by 5×10 irradiated PBL (30 Gy) in triplicate in 96-well U-shaped microtitre plates. MoAbs (BT563, 33B3.1 and anti-Tac) were added on day 0 to 6 after the start of the MLR. Proliferation was measured after 8 h of ^{3}H-thymidine (1 µCi/well) incubation. The effect of the MoAbs was determined as the percentage of inhibition of proliferation in the MLR.

In vivo experiments

BT563 was given to 16 allograft recipients (5 heart and 11 kidney grafts) for rejection prophylaxis as part of their sequential treatment regimen also comprising cyclosporine and prednisone. BT563 was given on days 0–6 (seven doses). The daily dose was 5 mg in seven patients and 10 mg in the other nine. Trough levels were measured

during the course of BT563 and during the following week. Before, during and after treatment peripheral blood mononuclear cells were isolated. Fluorescence analyses were performed on a flow cytometer (Facscan; Becton Dickinson). Cells were stained with fluorescein (FITC) or phycoerythrin (PE)-conjugated WT31 (anti-T-cell receptor $\alpha\beta$ complex) and/or 2A3.

Results

In monitoring the effect of BT563 on peripheral blood it is essential to use an anti-IL-2R MoAb that recognizes another epitope of the IL-2R. In the flow cytometric analyses we initially used unconjugated BT563 with FITC-labelled goat anti-mouse serum (GAM-FITC) as a second step, and PE conjugated 2A3. With this technique 2A3-PE appeared to be bound to free binding sites of the goat anti-mouse (GAM) antibodies in the BT563 GAM-FITC complex, making it unsuitable for monitoring the effect of BT563. By direct labelling of BT563 with FITC this problem was solved. Anti-Tac binds to the IL-2R, after which administration of 2A3 does not result in further binding. Apparently the epitope for 2A3 on the IL-2R is covered by anti-Tac. In contrast, after incubation with BT563-FITC the 2A3-PE is bound to the IL-2R, suggesting that 2A3 recognizes another epitope on the IL-2R that is not covered by prior BT563 binding. This makes 2A3 a suitable agent for monitoring peripheral blood cells in BT563-treated patients.

In vitro experiments

Increasing concentrations of BT563 were first tested for their ability to inhibit proliferation in the MLR. As shown in Fig. 1 lymphocyte proliferation was inhibited in a dose-dependent manner with maximal (86%) inhibition at concentrations above 500 ng/ml. The inhibition obtained with anti-Tac, also shown in Fig. 1, was considerably less, with a maximal inhibition of 42.5%. Results with 33B3.1 were similar to those with BT563.

If the MoAb was added to the MLR on day 1, 2, 3 or 4 BT563 gave a maximal inhibition, even if added on day 4. Later addition no longer inhibited, proliferation to this extent (Fig. 2). Unlike BT563, addition of anti-Tac on day 4 did not result in inhibition to the same extent as on day 0 (42.5% → 20%). Again with 33B3.1 we found an inhibitory effect similar to that with BT563.

In vivo experiments

BT563 administration did not elicit any significant side effects. In peripheral blood samples, collected 1 h after the first dose of BT563 anti-IL-2R positive cells could no longer be detected. Blood samples collected before each subsequent dose of BT563 remained negative for IL-2R$^+$ cells during treatment. After treatment these cells remained undetectable until day 9 (range 4–5) after the last

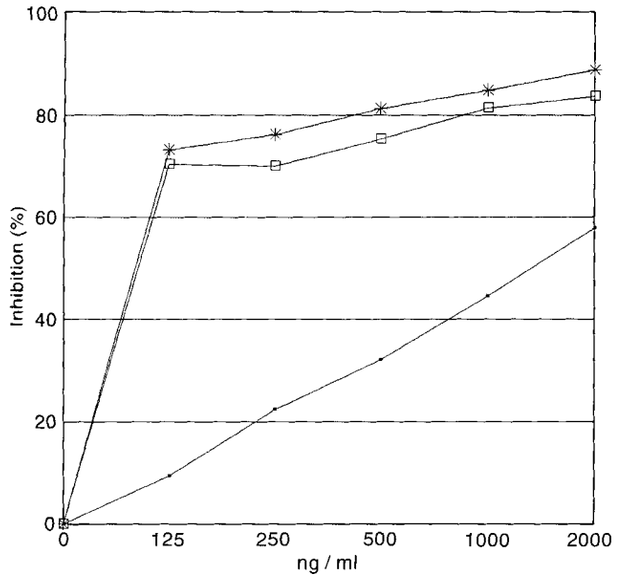

Fig. 1 Inhibition of lymphocyte proliferation in the MLR by increasing concentrations of BT563 (*), 33B3 (□) and anti-Tac (■)

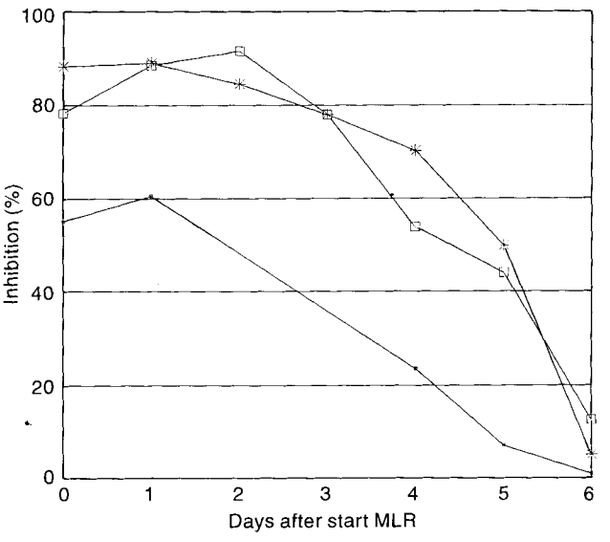

Fig. 2 Inhibition of lymphocyte proliferation in the MLR by 2000 ng/ml BT563 (*), 33B3 (□) and anti-Tac (■) in relation to the day of addition after the start of the MLR

dose. During treatment the numbers of $CD4^+$, $CD8^+$ and $WT31^+$ cells did not change significantly. Trough levels of BT563 reached a plateau after day 4. The trough levels on day 6 ranged from 1063 to 4572 ng/ml (mean 2619 ng/ml) in the 5-mg group and from 4357 to 8870 ng/ml (mean 6107 ng/ml) in the 10-mg group.

Discussion

BT563 is a murine IgG1 anti-IL-2R MoAb directed against the p55 subunit of the IL-2R. This MoAb is able to induce an 86% inhibition of MLR proliferation and was shown to be more potent than anti-Tac (42.5% inhibition). Even if administered after 4 days of culture BT563 was able to give a similar degree of inhibition as on immediate addition to the culture medium. In contrast the administration of anti-Tac after 4 days resulted in only 50% of the initial inhibitory effect. Both experiments using 33B3.1 gave results similar to those with BT563.

In peripheral blood samples $IL\text{-}2R^+$ cells were not detected from 1 h after the first dose until 8 days after the last dose. A similar pattern was found by Lemauff et al. during treatment with 33B3.1 [5]. However, anti-Tac administration was shown not to result in the disappearance of IL-2R expression on circulating T-cells [2]. Whether these striking differences in the effects of BT563 and anti-Tac on peripheral blood reflect differences in rejection prophylaxis potency is unknown. The similarity of the effects of BT563 and 33B3.1 in the in vitro experiments as well as on peripheral blood suggests an equal immunosuppressive potential.

Clinical tolerance of BT563 was excellent. ATG and OKT3 are both known for their sometimes life-threatening side effects. BT563 appears to be free of these problems.

The results we have obtained so far suggest that BT563 is an effective inhibitor of IL-2R-mediated lymphocyte activation. The clinical value of this new immunosuppressive drug is currently under investigation.

References

1. Herve 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:1072–1073
2. Kirkman RL, Shapiro ME, Milford EL, Tilney NL, Waldmann TA, Zimmerman CE (1989) Early experience with anti-Tac in clinical renal transplantation. Transplant Proc 21:1766–1768
3. Kirkman RL, Shapiro ME, Carpenter CB, McKay DB, Milford EL, Ramos EL, Tilney NL, Waldmann TA, Zimmerman CE, Strom TB (1991) A randomized prospective trial of anti-Tac monoclonal antibody in human renal transplantation. Transplantation 51:107–113
4. Kupiec-Weglinski JW, Diamantstein T, Tilney NL (1988) Interleukin-2 receptor targeted therapy – rationale and applications in organ transplantation. Transplantation 46:785–792
5. Lemauff B, Gascan H, Olive D, Moreau JF, Mawas C, Souilliou JP, Jacques Y (1987) Parameters of interaction of a novel monoclonal antibody (33B3.1) with the human interleukin-2 receptors: interrelationship between 33B3.1, anti-Tac, and IL-2 binding sites. Hum Immunol 19:53–68