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# Redundancy of the cytokine network in the development of rejection after clinical heart transplantation

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**Abstract** We used reverse transcriptase–polymerase chain reaction analysis to study the effects of anti-rejection prophylaxis with an anti-interleukin (IL)-2 receptor (IL-2R) monoclonal antibody (BT563) on the allogeneic process by analyzing intragraft IL-2, IL-4, and IL-15 mRNA expression. Analysis showed an association between rejection and intragraft IL-2 mRNA and IL-4 mRNA transcription, whereas IL-15 was consitutively expressed: IL-2 62% (8/13) during rejection versus 23% (8/35) during immunological quiescence ($P < 0.01$); IL-4 69% versus 23% ($P < 0.01$). BT563 therapy influenced the intragraft mRNA expression of IL-2 and IL-4 but not of IL-15. In endomyocardial biopsies (EMB) showing rejection, mRNA expression of IL-2 was detectable in 40% (2/5) during BT563 treatment versus 75% (6/8) in the absence of BT563; for IL-4, 23% versus 88%, respectively. In contrast, IL-15 mRNA transcription was not affected. Quantitative analysis in rejection EMB showed comparable IL-15 mRNA levels during and after BT563 treatment. This study demonstrates that therapeutic intervention within the IL-2-dependent T-cell activation cascade does not completely prevent rejection. Other cytokines, such as IL-15, may participate in IL-2-independent rejections.

**Key words** Anti-interleukin-2 receptor monoclonal antibodies · Prophylaxis · Interleukin-15 · Redundancy

## Introduction

Immune responses towards allografts are mediated by cytokines secreted by activated T-lymphocytes and macrophages. After clinical heart transplantation, an association between acute rejection and intragraft interleukin (IL)-2 mRNA expression has often been reported [1–3]. In view of the importance of IL-2 in the cardiac rejection process, blockade of the IL-2 receptor (IL-2R) by monoclonal antibodies may provide an opportunity to interrupt this T-cell-mediated immune response. The IL-2R is a multimeric complex of three transmembrane proteins ($\alpha$, $\beta$, $\gamma$), of which the $\alpha$-chain only is expressed by activated T-cells. Apart from IL-2, the $\beta$- and $\gamma$-chains of the IL-2R also interact with other potent T-cell growth factors such as IL-4 and IL-15 [4]. In the present study, we have used anti-IL-2R monoclonal antibodies in an attempt to prevent acute rejection after clinical heart transplantation. Patients were treated with the mouse anti-IL-2R monoclonal antibody, BT563, directed against the $\alpha$-chain, for 12 days in combination with cyclosporine and prednisolone. To monitor the in vivo effect of BT563 on the T-cell activation cascade, we measured intragraft IL-2, IL-4, and IL-15 mRNA expression in endomyocardial biopsies (EMB) with and without histological signs of acute rejection.

## Patients and methods

### Subjects

Patients were transplanted between February 1996 and November 1996 and received rejection prophylaxis with a mouse anti-IL-2R monoclonal antibody (BT563; Biotest Pharma, Dreieich, Germany), directed against the α-chain of the IL-2R complex. BT563 was given in a dosage of 11 mg i.v. from day 0 to day 12 after transplantation. Cyclosporine was started at day 0. Rejection was histologically diagnosed in EMB using the criteria of the International Society for Heart and Lung Transplantation [5]. For the present study, we evaluated the effect of BT563 on intragraft IL-2, IL-4, and IL-15 using reverse transcriptase–polymerase chain reaction (RT–PCR) analysis. From 15 patients, serial EMB ($n = 48$) were taken weekly until the first rejection episode or until day 42 post-transplant for non-rejecting patients.

### Qualitative and quantitative mRNA analysis by RT–PCR

Using techniques previously reported in detail [1, 3, 6], total RNA was extracted from snap-frozen EMB. cDNA was synthesized from the isolated mRNA with random primers. Aliquots (representing 1/20 EMB) were directly used for PCR amplification using sequence-specific primers for IL-2, IL-4, IL-15, and β-actin. Verification of an RT–PCR product was achieved by Southern blot hybridization. To estimate the relative initial amount of functional IL-15 mRNA in EMB, a competitive RT–PCR assay was used and comparison was made against the housekeeping β-actin gene. To obtain a standard curve for IL-15 and β-actin mRNA, serial dilutions of specific internal control fragments of known amount were coamplified. These internal controls could be analyzed after amplification by their smaller size by agarose gel electrophoresis. The intensity of the band was measured with a DC-40 camera (Kodak, Rochester, N.Y., USA) and analyzed using Kodak electrophoresis analysis software to determine the equilibrium between target and internal control. Corrections for the expression of the β-actin gene were made for the amount of mRNA used for reverse transcription and the efficacy of each reaction.

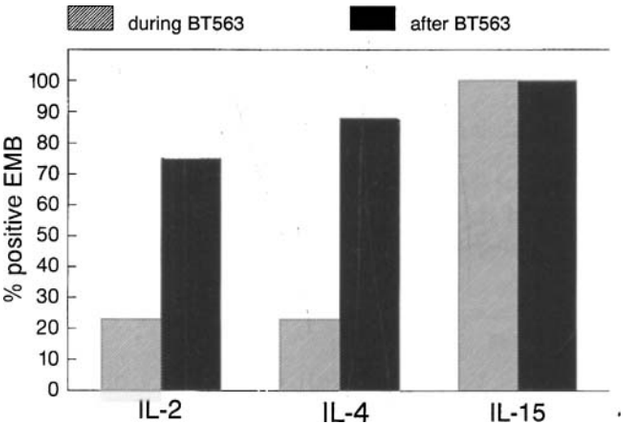


**Fig. 1** Data are expressed as the percentage of rejection endomyocardial biopsies *(EMB)* containing mRNA for interleukin *(IL)*-2, IL-4, and IL-15. BT563 prophylactic anti-rejection therapy clearly affected the intragraft mRNA expression of IL-2 and IL-4, whereas IL-15 was present in all rejection EMB

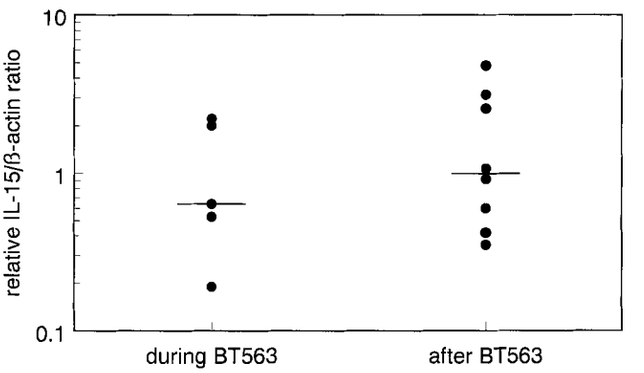


**Fig. 2** IL-15 mRNA levels are depicted relative to those of β-actin. Quantitative reverse transcriptase–polymerase chain reaction analysis showed comparable intragraft IL-15 mRNA levels in rejection EMB during and after BT563 treatment

## Results

The presence of intragraft mRNA expression of both IL-2 and IL-4 but not of IL-15 was associated with acute rejection. In 62% (8/13) of the rejection EMB, IL-2 mRNA expression was detectable versus 23% (8/35) in no-rejection EMB ($P < 0.01$, Fisher's exact test); IL-4 88% versus 23% ($P < 0.01$). Messenger coding for the IL-15 gene was constitutively expressed. Distinct intragraft mRNA profiles were found between rejection EMB during BT563 or after BT563 treatment (Fig. 1). During BT563 therapy, mRNA expression of IL-2 was detectable in 40% (2/5) of the rejection EMB versus 75% (6/8) after BT563 treatment. For IL-4 these percentages were 40% and 88%, respectively. IL-15 mRNA expression was found in all rejection EMB, irrespective of BT563 treatment. Therefore, we performed quantitative RT–PCR analysis in order to detect an in vivo effect of BT563 on intragraft IL-15 mRNA levels. However, using the competitive template RT–PCR, we found comparable IL-15 mRNA levels in rejection EMB during and after BT563 treatment (Fig. 2).

## Discussion

In the present study, we have demonstrated that anti-rejection induction therapy with the mouse anti-IL-2R monoclonal antibody, BT563, significantly influences mRNA expression of IL-2 and IL-4 associated with rejection. In contrast, no effect of BT563 induction therapy on the T-cell growth factor, IL-15, was found. Although anti-IL-2R monoclonal antibodies are capable

of blocking the IL-2-dependent T-cell activation cascade in vivo, acute rejections still occur. This observation suggests that cardiac allograft rejection can be mediated by growth factors other than IL-2 or IL-4. IL-15 and IL-2 have overlapping biological functions. IL-15 mediates its function through the $\beta$- and $\gamma$-chains of the IL-2R and its own IL-15$\alpha$ chain [4]. In contrast to IL-2 and IL-4, IL-15 is produced by macrophages and other non-T-cells. During cardiac rejection, these macrophages are abundantly present within the graft, suggesting that IL-15 can participate in this process. Studies in IL-2 knock-out mice confirm this assumption [7]. Recently, a role for IL-15 in the immune response after clinical kidney and liver transplantation was also reported [8, 9]. In conclusion, our data suggest that IL-15 may have overtaken the function of IL-2 during cardiac allograft rejection when the IL-2 pathway is blocked by anti-IL-2R monoclonal antibodies.

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