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Prolongation of heart allograft survival in rats by interferon-specific antibodies and low dose cyclosporin A

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Interferons (IFNs) are important cytokines which exhibit antiviral, antitumor, anticellular, as well as immunoregulatory activities [1]. Among these multiple activities, IFNs are potent inducers of MHC antigen expression of a great variety of cells [2–4], helper and maturation factors in B-cell antibody production [5], and macrophage function [6]. IFNs may therefore play a critical role in triggering antigen recognition and allograft rejection.

Cyclosporin A (CyA) is a potent immunosuppressor which selectively inhibits helper T-lymphocyte proliferation in response to alloantigen presentation [7, 8]. CyA has been reported to inhibit interleukin 2 and IFNγ production by helper T lymphocytes [9–11]. In addition, CyA may induce monocyte production of prostaglandin E2 [12], which then reduces MHC class II expression on endothelial cells, monocytes, and macrophages [13].

However, the clinical use of CyA is plagued by its toxic (in particular nephrotoxic) side-effects. These toxic effects are clearly dose-related. It may be very important to develop new products which can act synergistically with CyA to inhibit lymphokine production. The aim of this study was to investigate the effects of combined IFN-specific antibodies and low dose CyA on cardiac allografts in inbred strains of rats.

Key words: Immunosuppression – Cytokines – Interferons – Cyclosporine A

Material and methods

Adult male Lewis (Lew, RT1l) and brown Norway (BN, RT1n) rats weighing 200–250 g were purchased from CNSEAL (Orleans La Source, France). Rats were operated on under clean but not sterile conditions. Heterotopic intraabdominal heart transplantations were carried out according to the technique of Ono and Lindsey [14]. CyA (Sandimmune, Sandoz, Basel) was administered to rate by gavage via an orogastric tube. Polyclonal sheep antimouse IFNα/β, prepared and purfied as described previously [15], had a neutralizing titer of 6.4×10^6 against 8 units of mouse IFNα/β when assayed on mouse L929 cells [16] and a titer of 2000 against 5 units of rat IFNα/β. Polyclonal sheep antirat IFNγ was a gift of H. Schellekens (Rijsnijk, The Netherlands) and had a neutralizing titer of 8000 against 16 units of rats IFNγ. These antibodies were administered intravenously via the dorsal penile vein. In the BN into LEW combination, heart allografts were performed, and 5 experimental groups of 10 rats each were constituted. In group 1, rats were treated by 0.5 ml of polyclonal sheep antirat IFNγ antibodies on days −4, −1, +2, +5, +8, and +11. Rats of group 2 were treated by 0.5 ml of polyclonal sheep antimouse IFNα/β antibodies on the same day schedule. In group 3, anti-IFNγ therapy was associated with low dose CyA (2.5 mg/kg body weight on day 0 and then every day). Group 4 consisted of rats treated only by CyA (2.5 mg/kg daily) and group 5, of heart allografts. The survival time of heart allografts was assessed by daily palpation. Rejection time was the moment of cessation of beating. At that time, the recipient was autopsied to detect technical complications. Heart allografts were excised and processed for conventional and immunohistochemical examination. A peroxidase-antiperoxidase method using mouse monoclonal antibodies to rat MHC antigens (F 16.4.4 for class I and MRC 0 × 17 for class II MHC Ag) was carried out as previously described [17]. Results were expressed as mean survival time ± standard error of the mean (MST ± SEM). Student's *t*-test was used for comparing the means of the different groups.

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Results

In the BN into LEW combination of inbred rats (Table 1), the survival of heart allografts was significantly ($P < 0.05$) prolonged in rats of group 1 (treated by anti-INFγ antibodies; mean survival time, MST ± SEM = 9.7 ± 0.2 days), in rats of group 2 (treated by anti-IFNα/β antibodies; MST ± SEM = 8.0 ± 0.2 days), and in rats of group 4 (treated by low dose CyA; MST ± SEM = 9.4 ± 0.2 days) when compared with control heart allografts (group 5; MST ± SEM = 6.8 ± 0.7 days). The observed prolongation was more important when CyA was associated with anti-IFNγ antibodies (group 3; MST ± SEM = 14.6 ± 0.4 days). Immunohistochemical analysis of the control heart allograft revealed induction of the expression of MHC class I antigens on the myocardial cells. To the contrary, no expression was observed on the myocardium of anti-IFN-treated heart allografts.

Table 1. Rejection time of BN cardiac allografts into LEW recipients

Group	n	Graft survival (days)	MST ± SEM (days)
1 (Anti-IFNγ)	10	8, 9, 9, 10, 10, 10, 10, 10, 10, 11	9.7 ± 0.2*
2 (Anti-IFNα/β)	10	7, 7, 8, 8, 8, 8, 8, 8, 9, 9	8.0 ± 0.2*
3 (Anti-IFNγ + CyA)	10	12, 13, 14, 14, 15, 15, 15, 16, 16, 16	14.6 ± 0.4**
4 (CyA)	10	8, 9, 9, 9, 9, 9, 10, 10, 10, 11	9.4 ± 0.2*
5 (Control)	10	6, 6, 6, 7, 7, 7, 7, 7, 7, 8	6.8 ± 0.7**

* Difference between groups 1, 2, 4 significant ($P < 0.05$) and ** between groups 3 and 5 significant ($P < 0.001$) BN, brown Norway rats; LEW, Lewis rats; MST, mean survival time

Discussion

IFNγ is mainly released by helper T lymphocytes upon activation by alloantigens and is a critical cytokine which is a potent inducer of MHC antigen expression on a great variety of cells [2–4] and an activator of monocyte-macrophage functions [6]. Previous studies have demonstrated that antibodies to IFNγ may delay allograft rejection [18, 19].

Our results confirm these studies and show that low dose CyA and antibodies to IFNγ may act synergistically to delay heart allograft rejection in the rat. In fact, CyA has been shown to inhibit lymphokine production [9–11] and in particular to reduce IFNγ production in mice [11] and in man [10]. In addition, CyA may reduce cytokine production by macrophages [20] and class II MHC Ag expression on macrophages [12, 13]. In our study, we observed the absence of the induction of expression of MHC class I antigens on rejected myocardial cells after treatment by antibodies to IFN. In conclusion, IFNγ-specific antibodies may represent an useful reagent to potentiate low dose CyA therapy, probably by MHC expression down-regulation.

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