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Changes in blood lymphocyte populations in experimental bowel allograft rejection

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Abstract The aim of this study is to measure percentages of lymphocyte populations and IL-2R cellular expression in peripheral blood during the rejection of a small bowel allograft (SBA) in the rat. Thirty rats were allotted to three groups: A, control, no transplantation (Tx); B, rats receiving an orthotopic SBA; C, similar SBA but with thymostimulin (TP-1) administered before Tx, aimed at increasing the intensity of and accelerating rejection. The percentages of CD19, CD5, CD4 and CD8 cells and of IL-2R were determined when rejection was present. Rejection appeared in rats in group B between days 11 and 26 post-Tx and in group C between days 6 and 7 post-Tx ($P < 0.001$). In both B and C groups, CD5 and CD4 cells decreased ($P < 0.005$) and CD8 cells increased ($P < 0.001$). A correlation between CD8 and IL-2R content was found ($P < 0.05$). In group C, earliness of rejection correlated with the percentage of CD8 cells ($P < 0.05$) and the intensity of rejection with numbers of CD8 and CD19 cells ($P < 0.05$).

Key words Lymphocyte · populations · IL-2R · Rejection · Bowel allograft

Introduction

Until recently, the immune response in allograft transplantation, particularly in bowel allograft transplantation, has not been understood because of its complexity. It is well known that thymus-derived lymphocytes (T-cells) play a major role in transplant rejection; in small intestine allografts the large quantity of lymphoid tissue may stimulate a vigorous rejection from the recipient, and may also induce graft-versus-host disease (GVHD) from the graft. At present it is known that many immunosuppressive regimens to prevent either rejection or GVHD, among these cyclosporin and FK506, work by inhibiting IL-2 mRNA synthesis, blocking early lymphocyte activation. Dynamic lymphocyte population changes and the expression of IL-2 receptors by lymphocyte subsets during the rejection are, however, still not fully understood. The aim of the present study is to ascertain the percentage changes of lymphocyte population and subpopulations and the expression of IL-2R on them, in peripheral blood, during the rejection of an orthotopic small bowel allograft.

Materials and methods

Animals

Wistar Furth rats were allotted to three groups: group A (10 rats), control group without transplantation (Tx); group B (10 rats) received an orthotopic small bowel allograft (SBA); group C (10 rats) received an orthotopic SBA and a thymic hormone [thymostimulin (TP-1) 2 mg/kg i.m.] for 3 days before Tx, aimed at increasing the intensity of and accelerating the rejection. Inbred Fisher male rats were employed as donors and Wistar Furth male rats as recipients; this endogamic strain of rats is directed to produce a single form of rejection. The rats weighed between 200 and 350 g. All the animals were delivered by a laboratory (IFFA, Lyon, France) under standard conditions. All rats were kept under the same conditions in metabolic cages and received paired feeding.

Table 1 Number of rats in groups B and C according to the intensity and earliness of the rejection

	Rejection intensity (pathological criteria)		Maximal clinical signs of rejection			
	(++)	(+++)	6th day	7th day	11th–17th day	17th–26th day
Group B	6	4	0	0	6	4
Group C	8	2	2	8	0	0

Table 2 Preoperative values of CD5, CD4, CD8, CD19 und IL-2R (1 week before transplantation: groups A, B, C; on the day of surgery: group A)

	CD5 (%)	CD4 (%)	CD8 (%)	CD19 (%)	IL-2R (%)
Group A	71.7 ± 1.49	43.5 ± 2.39	23.5 ± 1.91	18.1 ± 1.43	1.7 ± 3.3
Group B	70.5 ± 2.10	42.6 ± 1.17	24.3 ± 2.10	16.4 ± 1.57	1.3 ± 3.4
Group C	72.3 ± 1.23	45.7 ± 2.80	23.8 ± 2.60	15.7 ± 1.36	1.7 ± 2.9
Group A (surgery day)	71.9 ± 1.45	44.9 ± 2.30	22.9 ± 1.80	17.2 ± 1.30	1.9 ± 3.1

Operative procedures

Donors were fasted for 24 h with water ad libitum. All procedures were performed under anaesthesia using an i.m. dose (2 ml/kg) of a mixture of ketamine (0.5 mg/ml), diazepine (2 mg/ml) and atropine (0.1 mg/ml). After anaesthesia, rats received one dose of 1000 IU penicillin-G and 0.5 mg of gentamicin, undergoing surgery according to an experimental model previously described by our team [8] based on an experimental small bowel orthotopic transplant technique described by Monchick and Russell in 1971 [13]. In the donor, the small bowel was dissected from the colonic and vascular attachments and the graft removed with an aortic cuff joined to the superior mesenteric artery. As the warm ischemia time was very short the graft was not perfused before the extraction. After removing the graft it was perfused ex vivo with cold Ringer lactate solution through the aortic cuff. Vascular end-to-side porta-caval and mesenteric-aortic anasthomoses were carried out using a double-running 9/0 monofilament suture. Once the vascular clamp was removed and revascularisation of the graft considered to be correct, the recipient's small bowel was removed and a double intestinal anasthomosis, an end-to-end running everting suture using 7/0 silk, was performed, placing the graft orthotopically.

After anaesthesia, rats received one dose of 1000 IU penicillin-G and 0.5 mg of gentamicin. After operation the rats received a normal diet and water ad libitum and they received no antibiotics in the postoperative period. The rats were weighed 6 times a week, and were sacrified when they showed the clinical signs of rejection described by Schraut and Lee [20]: weight loss, profuse diarrhoea and lack of redding of nose, ears and feet. A histological study of the grafts was carried out by a pathologist who was unaware of the origin of the samples and the results were graded as: (i) incipient rejection (+): normal appearance of villi and moderate mononuclear cell infiltration; (ii) moderate rejection (++): great mononuclear cell infiltration but moderate destruction of villi; or (iii) massive rejection (+++): total destruction of villi and great mononuclear cell infiltration.

One week before the surgical procedures, blood was drawn from a rat tail incision by pressing out the blood. On the day of surgery a blood sample was obtained from one rat of the control group, but no blood was obtained from animals undergoing surgery to avoid lymphocyte changes due to blood loss. In addition, blood samples were obtained from rats of groups B and C on the day of sacrifice. In blood samples, CD5+, CD4+, CD8+ and CD19+ cell percentages and IL-2R percentages on the cellular surface were determined by flow cytometry (FACS, Becton-Dickinson) using monoclonal antibodies (mAb; anti-rat CD5, CD4, CD8, CD19, and IL-2R provided by Sera-lab): A 100-µl sample of blood with heparin was incubated with 10 µl of the corresponding mAb for 10 min at 10 °C. Subsequently, cells were lysed by means of Immunopred (phormic acid, sodium carbonate, paraformaldehyde solution) and were fixed and identified by flow cytometry.

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Cell and IL-2R percentages were compared 1 week before the surgical procedure between groups A (control), B and C; on the day of surgery between the control group and samples from groups B and C obtained 1 week before; and on the day of sacrifice, between groups A (control), B and C (rejection). Data were analysed by means of Student's and ANOVA tests. Regression and Kendall correlation tests were used to study any relationship between variables, as well as between variables and the precocity and intensity of the rejection. Differences and correlation were considered significant at the $P < 0.05$ level.

Results

Rejection appeared in rats in group B between the 1st and 26th post-transplantation days ($\bar{x} = 17.7 \pm 1.8$) and in group C between days 6 and 7 ($\bar{x} = 6.7 \pm 0.1$); this difference was statistically significant ($P < 0.001$). The intensity of rejection was moderate (++) or massive (+++) in all cases. Table 1 shows the number of rats in each group with (++) or (+++) intensity and the time of rejection. No rats showed GVHD.

No differences were found between the percentages of CD5, CD4, CD8 or CD19 cells nor IL-2R from the control and groups B and C obtained 1 week before the surgical procedure as well as between those samples and the sample obtained from the control group on the day of surgery (Table 2). CD5 and CD4 cell percentages decreased in groups B and C when rejection was present

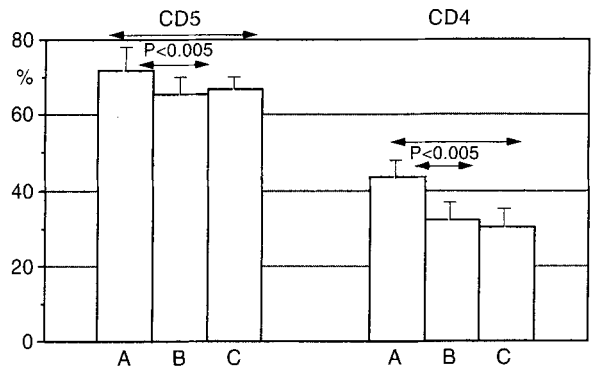

Fig. 1 CD5 and CD4 cell percentage variations in small bowel allograft rejection. Differences between control (group A), transplantation (group B) and transplantation plus thymostimulin (group C)

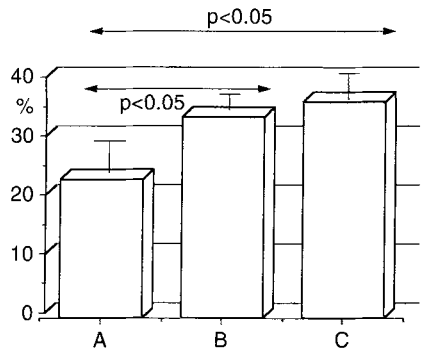

Fig. 2 CD8 cell percentage variations in small bowel allograft rejection. Differences between control (group A), transplantation (group B) and transplantation plus thymostimulin (group C)

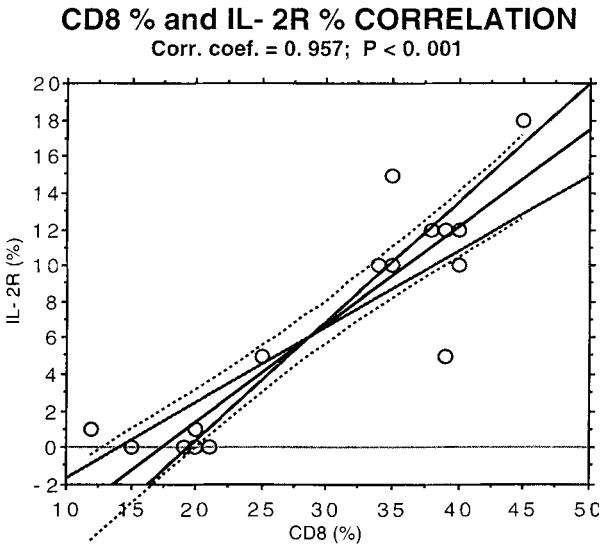

Fig. 3 Correlation between CD8 cell percentage and IL-2R expression

(Fig. 1), whereas CD8 cell percentages increased at the same time (Fig. 2).

The IL-2R percentage increased in both B and C groups on rejection, but in group C the increase was greater (Table 3). CD8 cell and IL-2R percentages showed a significant correlation (Fig. 3). The IL-2R percentage also showed a relationship with that of CD19 (correlation coefficient = 0.623; $P = 0.05$; Kendall test), however, the CD19 variation was not statistically significant in groups during rejection. In group C, the earliness and the intensity of rejection were directly correlated with activated CD8 and CD19 cell percentages (Table 4).

Discussion

Because it is known that antigens from the allograft are presented to the CD4+/T-helper cells by means of antigen-presenting cells, it is believed that the recipient immune response starts with the host T-helper cells against graft passenger leucocytes which express high levels of major histocompatibility complex (MHC) class II [12]. The antigen-presenting cell, in addition to processing the antigen, secretes IL-1, which is essential for T-cell proliferation. IL-1 has pro-inflammatory effects: promoting B-cell growth and differentiation, a synergic effect with IL-4 and IL-6, increasing the cytotoxicity of natural killer cells, increasing the elaboration of platelet activation factor by macrophages and also chemotactic effects on macrophages [4, 9, 19]. The activated T-helper cell releases IL-2 (previously called T-cell growth factor). Until recently, IL-2 has been considered as the main cytokine in rejection because it stimulates the differentiation and proliferation of T cytotoxic cells (CD8+ cells), which are the main effector cells in rejection. In addition, however, IL-2 has an autocrine effect of augmenting, IL-2 and other lymphokine production by activated T-helper cells. IL-2 also has the effect of promoting the recruitment and proliferation of new CD4+ cells and activation factors (such as IL-4 and IL-6). In addition, IL-2 promotes the release of gamma interferon as well as tumour necrosis factor and other cytokines [5, 10, 11, 16]. The increase of IL-2 receptor percentages has been reported during the rejection of different organs [3, 18] and the measurement of soluble IL-2R has been proposed as an indicator of liver allograft rejection [12].

Our results show an important increase in IL-2R when clinical signs of rejection are present and, in addition, show an increase of CD8 cells and also an increase of IL-2 receptors.

CD8 is expressed by cytotoxic/suppressor cells which preferentially respond to antigens presented in conjunction with MHC class I molecules. Activation of CD8+ specific cytotoxic T-lymphocytes by allogenic class I

Table 3 Pre- and posttransplantation values of IL-2R

	Pretransplantation (%)	Posttransplantation (%)
Group A	1.7 ± 3.3	——
Group B	1.3 ± 3.4	8.8 ± 2.5 ($P < 0.05$)
Group C	1.7 ± 2.9	10.7 ± 1.7 ($P < 0.001$)
Group A (surgery day)	1.9 ± 3.1	——

Table 4 Correlation between the intensity and earliness of the rejection and the increase of CD8 and CD19 cells in group C (Kendall correlation coefficient)

Intensity of rejection	CD8 $P = 0.0199$
Intensity of rejection	CD19 $P = 0.0223$
Earliness of rejection	CD8 $P = 0.0199$
Earliness of rejection	CD19 $P = 0.0223$

and their stimulation by IL-2 causes direct specific foreign cell lysis [2], perhaps through direct contact by means of perforin [21].

This study also demonstrates a correlation between activated CD8 cells and the intensity and precocity of rejection when the host immune response is stimulated by means of a thymic hormone [thymostimulin (TP-1)]. Previous studies showed that thymostimulin was able to inhibit the decrease of the immune response induced by both anaesthesia and surgical trauma in the rat [6, 15], by increasing the CD4 cell percentage and inhibiting the increase of the CD8 cell percentage. However, when rats receiving thymostimulin also underwent transplantation of a small bowel allograft, we observed an increase of CD8 cell content in the spleen, thymus and intestinal lymphatic nodes, a decrease of CD4 cells in the spleen and an accelerated rejection [7]. The importance of CD8 cell changes during cardiac allograft rejection has been exposed by Carlquist et al. [1] in three murine experimental models. These authors found a significantly increased percentage of CD4 and a significantly decreased percentage of CD8 cells infiltrating grafts when allografts had a long survival but, in contrast, an increase of CD8 and a decrease of CD4 infiltrating graft cells when allografts were rejected in a short posttransplantation period. In the small bowel rejection of a fully allogeneic graft in rats, Oberhuber et al. [7] found intraepithelial lymphocytes. Among these, 45 % were neither CD4+ nor CD8+, 46.4 % were CD8+ cells, 8 % were CD4+ cells and 23 % were infiltrating host lymphocytes. This observation may be important in the consideration of the role of CD8 lymphocyte subsets in small bowel allograft rejection, especially with regard to passenger leucocytes from the graft.

In our study, in the group with thymostimulin treatment, a relationship was found between the percentage of activated CD19 cells and the intensity of rejection. B-cells are also activated by cytokines produced by T-cells (IL-3, IL-4, IL-6, IL-7, IL-13, BCDF, BCAF, BCSF) [16] in the presence of the antigen to produce high levels of anti-class I antibodies. Even though this is not very important in acute rejection [14], it has an important role in accelerating vascular rejection [2].

We believe that our paper shows the importance of CD8 cells in the rejection phenomenon, particularly in small bowel transplantation. In addition, the increases of CD8 cells and IL-2R in peripheral blood should be considered as a diagnosis of rejection. Finally, we agree with P.J. Morris [14] in believing that therapeutic options, including monoclonal antibodies against the IL-2 receptor, may be the future to controlling small bowel allograft rejection.

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