

## Short reports

# Immunohistological study of distribution of $\gamma\delta$ lymphocytes after allogeneic bone marrow transplantation

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### Abstract

The distribution of T lymphocytes expressing the  $\gamma\delta$  form of the T cell receptor was studied in the liver, intestine, and major lymphoid organs, after bone marrow transplantation (BMT), including cases of graft versus host disease (GvHD). The number of  $\gamma\delta$  as a proportion of the total number of CD3 positive cells did not differ from that found in normal tissues; the higher percentage normally found in the intestinal epithelium and splenic red pulp was maintained. This, and the results of a previous study undertaken on the skin, provide no evidence that  $\gamma\delta$  T cells have a particularly important role in T cell regeneration after marrow transplantation or in the pathogenesis of the epithelial lesions associated with GvHD.

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The T cell receptor (TCR) exists as an  $\alpha\beta$  or  $\gamma\delta$  heterodimer non-covalently linked to the CD3 complex of proteins.<sup>1</sup> In birds and mice most intraepithelial T lymphocytes in the gut and skin bear TCR $\gamma\delta$ , suggesting that they have a role in mucosal immunological surveillance.<sup>2,3</sup> In man the role of this subpopulation of T cells is not well defined. They account for about 5% of T lymphocytes in the blood and tissues, except for the intestinal epithelium and the splenic red pulp, where their numbers are somewhat greater.<sup>4</sup>  $\gamma\delta$  cells are commonly phenotypically immature (CD4 and CD8 negative), although CD8 and CD4 positive  $\gamma\delta$  cells occur in low numbers.<sup>1</sup> Increased numbers of  $\gamma\delta$  cells have been found in a variety of infections and immunological disorders.<sup>5</sup>

Acute GvHD following allogeneic bone marrow transplantation is characterised by damage to various epithelial surfaces, particularly of the skin, gut, and bile ducts, and occurs during a period of profound lymphodepletion produced by the pre-transplant conditioning. Increased numbers of CD3+, CD4-, CD8- cells have been reported in the peripheral blood during this period.<sup>6</sup> A previous study of post-transplantation skin in our laboratory showed the T cells infiltrating the epidermis in cutaneous GvHD to be of the  $\alpha\beta$  type, although a few of the dermal cells expressed  $\gamma\delta$  receptors.<sup>7</sup> This study was undertaken to extend this investigation by determining the

proportion of  $\gamma\delta$  lymphocytes after bone marrow transplantation in the major lymphoid organs and the other epithelia susceptible to GvHD.

### Methods

Eleven rectal biopsy specimens were obtained from 10 bone marrow allograft recipients for the investigation of diarrhoea 28 to 40 days (mean of 35 days) after transplantation. The patients had a mean age of 29 years and an underlying diagnosis of acute myeloid leukaemia (AML) in six cases, acute lymphocytic leukaemia (ALL) in two, chronic granulocytic leukaemia (CGL) in one and non-Hodgkin's lymphoma (NHL) in one. Seven biopsy specimens showed histological features of acute GvHD and four were normal. Normal large bowel tissue was taken from the unaffected resection margins of colectomy specimens containing carcinoma (two cases) and rectal biopsy specimens with normal histology, taken for the investigation of diarrhoea (two cases) from patients with a mean age of 50 years. Eight specimens of liver tissue from bone marrow allograft recipients of mean age 23 years, five AML, two ALL, one CGL, were obtained 30-420 days (mean of 120 days) after bone marrow transplantation, six during life by percutaneous needle biopsy, and two at necropsy. Five liver biopsy specimens showed GvHD; three were histologically normal. Seven necropsy specimens of normal liver from patients with a mean age of 53 years were used as controls. Necropsy specimens of lymph node, spleen, and thymus were obtained from 11 bone marrow allograft recipients of mean age 27 years, five AML, four ALL, two CGL, who died 20-480 days (mean of 112 days) after transplantation, six with and five without evidence of GvHD, and normal control tissues from necropsies on five patients of mean age 38 years, where the cause of death was unrelated to bone marrow transplantation. All necropsy material was taken within 24 hours of death.

All tissue samples were divided in half; one half was mounted in OCT compound, frozen in liquid nitrogen, and stored at  $-70^{\circ}\text{C}$ . The remainder was fixed in formalin and embedded in paraffin wax; 4  $\mu\text{m}$  sections were stained with haematoxylin and eosin for light microscopic examination. Serial frozen sections were cut at 5  $\mu\text{m}$  and labelled by the alkaline phosphatase-antialkaline phosphatase technique using monoclonal antibodies UCHT1 to

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Percentage of T lymphocytes bearing TCR $\gamma\delta$ 

Tissue	Normal controls	After transplantation	
		Non-GvHD	GvHD
<b>Rectum:</b>			
Epithelium*	8.3 (2.0-15.4)	4.5 (0-25.0)	5.4 (0-7.0)
Lamina propria*	1.6 (0.7-2.6)	2.6 (2.0-7.0)	1.5 (0-3.0)
<b>Liver:</b>			
Portal tracts	3.1 (0-10)	1.0 (0-4.7)	1.3 (0-4.3)
<b>Spleen:</b>			
Red pulp†	11.8 (5.0-21.0)	6.0 (3-6.7)	6.1 (0-16)
White pulp†	2.0 (0-3.0)	1.8 (0-2.5)	2.8 (0-8)
<b>Lymph node:</b>			
Thymus:	5.1 (2.0-10)	6.5 (1.3-11.1)	4.0 (0-7)
	3.3 (1.5-5.1)	0.3 (0-2.2)	1.3 (0-2.8)

Figures are medians with ranges in parentheses.

\*Epithelium > lamina propria  $p < 0.02$ .

†Red pulp > white pulp  $p < 0.05$ .

No other significant differences.

the CD3 glycoprotein complex (supplied by Professor P Beverley)  $\beta$ F1 to a framework region of the  $\beta$  chain of the human T cell antigen receptor, and TCR $\delta$ 1 to the  $\delta$  chain (T cell Sciences Ltd).

Morphometric analysis of each tissue section was undertaken using a semiautomated computer based image analysis system (Colour-morph, software supplied by Precision Instruments Ltd). This permitted measurements of epithelial length (mm) and areas ( $\text{mm}^2$ ) in lamina propria, portal tract, spleen, lymph node and thymus. The total numbers of cells stained with the three antibodies in these compartments were counted separately and the results expressed as the number of positive intraepithelial cells/mm epithelial length or cells/ $\text{mm}^2$ . The percentages of CD3 positive cells positive for TCR $\delta$ 1 and  $\beta$ F1 were calculated. Between 100 and 650 CD3 positive cells were counted in each section. Cells within biliary epithelium were very rare and were not quantified.

Statistical analysis was performed using the rank sum test.

## Results

In all cases the sum of TCR $\delta$ 1 positive and  $\beta$ F1 positive cells came close to the number of CD3 positive cells. The percentages of CD3 cells positive for TCR $\delta$ 1 are shown in the table. There was no significant difference in the percentage of  $\gamma\delta$  cells between normal controls and transplant recipients, with or without GvHD.

The percentage of CD3 cells positive for TCR $\delta$  was significantly greater in the epithelium of the rectum than the lamina propria and in the red pulp of the spleen than the white pulp. These differences were maintained in specimens after transplantation.

## Discussion

This study was undertaken to investigate the possible importance of  $\gamma\delta$  T lymphocytes after allogeneic bone marrow transplantation in the pathogenesis of GvHD and lymphocyte repopulation. The removal of T cells from the donor marrow has been shown to prevent the occurrence of GvHD, although the procedure is associated with an increased risk of graft rejection and leukaemic relapse.<sup>8</sup> Clearly, if the

development of GvHD were associated with a particular subset of T cells, it might be possible to prevent the disease without incurring the undesirable sequelae associated with the removal of all T lymphocytes.

The rationale for studying  $\gamma\delta$  lymphocytes in bone marrow recipients is that they seem to represent a more primitive or simple cell type which might be associated with early T lymphocyte repopulation. They have a more limited range of antigen specificities and are more numerous in certain animals, particularly in association with epithelial surfaces.<sup>2,3</sup> Most are CD4 and CD8 negative.<sup>1</sup> For several months after transplantation the lymphoid organs show profound depletion of lymphocytes and an increase in numbers of CD4, CD8 negative cells in the peripheral blood. The thymus is involuted and does not contain precursor T cells.<sup>9</sup> Impaired thymic processing might be expected to result in an increase of earlier, more immature cells or to extra-thymic maturation pathways, perhaps in the peripheral lymphoid organs.

In the previous study of post-transplantation skin undertaken in our laboratory, we showed that T cells infiltrating the epidermis were of the  $\alpha/\beta$  type, although a few of the dermal cells expressed  $\gamma\delta$  receptors.<sup>7</sup> This would not exclude a possible role for  $\gamma\delta$  cells in GvHD in other epithelial sites, or in the lymphoid organs which have been shown in experimental animals to be important sites in the initiation of the disease.<sup>10</sup>

In this study we have failed to show any significant change in the proportion of lymphocytes expressing  $\gamma\delta$  receptors after bone marrow transplantation and, indeed, the higher proportions normally found in the large intestinal epithelium and splenic red pulp were maintained. It thus seems unlikely that these cells have a particular role in immunological reconstitution after bone marrow transplantation or in the pathogenesis of GvHD.

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