J Clin Pathol 1990;43:461–464

Preferential localisation of human lymphocytes bearing γδ T cell receptors to the red pulp of the spleen

D Bordessoule, P Gaulard, D Y Mason

Abstract

About 4% of human T cells carry antigen receptor composed of γ and δ chains (rather than α and β chains). Double immunoenzymatic staining of frozen sections of 14 samples of human spleen showed that γδ bearing T cells were preferentially localised in the red pulp of this organ where on average they accounted for 17% of all T cells. There was no correlation between the number of γδ T cells and the diagnosis, with the exception of a case of malaria in which an unusually high number (40%) of T cells were of this type. The γδ bearing T cells were scattered randomly through the red pulp, and double staining combined with a marker of splenic sinusoids (CD36) showed that almost all lie outside the sinusoids within the cords of the red pulp. It is suggested that the double immunoenzymatic technique could be used for further studies of the prevalence of γδ bearing T cells in lymphocytic infiltrates.

The T cell receptor (TCR) for antigen is a heterodimeric molecule which exists in two alternative forms. In 90–99% of T cells the receptor is composed of α and β subunits. Cells expressing this type of TCR recognise peptide antigens in association with self-histocompatibility antigens. The remaining small minority of T cells expresses an alternative form of the receptor comprising γ and δ subunits.[1] In contrast to the major αβ-T cell population, which has a central role in recognising foreign antigens and thereby initiating both T and B cell immune responses, the function of the cell population expressing γδ is less clearly defined, because the rarity of these cells has made it difficult to define their antigen recognition repertoire.[2,3] Studies in this area have yielded differing results, including suggestions that γδ-T cells respond to mycobacterial antigens,[4,5] leishmaniasis,[4] tetanus toxoid,[6] immunoglobulin[7] and histocompatibility antigens.[8,9]

One approach to the assessment of the function of γδ T cells is to examine their distribution in different tissues by immunohistological techniques. In mice and birds this type of study has shown that most intraepithelial gut T lymphocytes and dendritic epithelial cells in the skin are of this type.[10–13] In a study of human tissues, however, we and others found no evidence of preferential localisation to these sites,[14,15] but we did note that the red pulp of the spleen seemed to contain more of these cells than other organs. In this paper we report a more detailed study of this phenomenon based on the analysis of 14 human spleens.

Methods

Frozen samples of human spleen were obtained from the histopathology departments of the authors' hospitals. The spleens had all been removed surgically and the diagnoses are shown in the table. Samples were snap frozen in liquid nitrogen and 5 μm cryostat sections cut. After air drying the sections were fixed in acetone for 10 minutes and then stored in foil as described previously.[16] Slides were warmed to room temperature and unwrapped before staining.

The monoclonal antibodies used in this study were directed against CD3, CD8 (both from Dakopatts), and CD36 (antibody IVC7, kindly provided by Dr A von dem Borne) and against TCR δ and β chains (reagents TCR δ and βF1 respectively, both kindly provided by Dr M Brenner). Anti-mouse Ig antibodies, both unconjugated and coupled to peroxidase or alkaline phosphatase, were obtained from Dakopatts a/s or from Dr K-J Pluzek. Alkaline phosphatase-anti-alkaline phosphatase (APAAP) immune complexes were prepared in one of the authors' laboratories. Reagents were diluted in TRIS-buffered saline (TBS) (0·5 M TRIS HCl, pH 7·6, diluted 1/10 with 0·15 M saline) containing 10% human serum, and TBS was also used for the washing steps.

Single labelling of antigens was carried out by the APAAP immunoalkaline phosphatase

Prevalence of T cells bearing γδ TCR in splenic red pulp

Case No	*Diagnosis*	*γδ-bearing cells/T cells (%)*
1	Reactive	18
2	Idiopathic thrombocytopenic purpura	18
3	Spherocytosis	22
4	Normal	20
5	Portal hypertension	6
6	Normal	9
7	Idiopathic thrombocytopenic purpura	21
8	Idiopathic thrombocytopenic purpura	12
9	Normal	18
10	Normal	13·5
11	Malaria	40
12	Idiopathic thrombocytopenic purpura	9
13	Normal	11
14	Normal	16
Mean		17
Range		6–40

Department of Haematology, University Hospital, Limoges, France
D Bordessoule

Department of Pathological Anatomy, CHU Henri Mondor, Creteil, France
P Gaulard

Department of Haematology, John Radcliffe Hospital, Oxford OX3 9DU
D Y Mason

Correspondence to:
Dr D Y Mason

Accepted for publication
4 January 1990

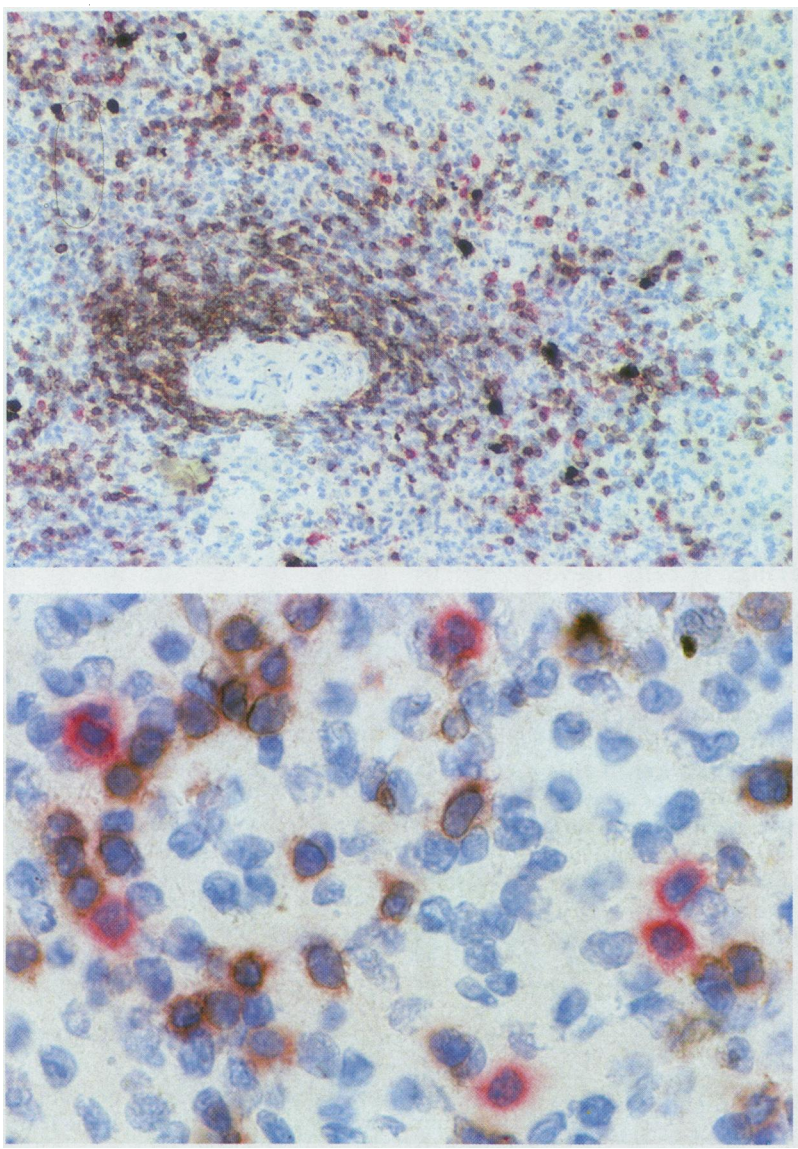

Figure 1 Double labelling of a frozen section of normal spleen with anti-TCR β antibody in brown (by immunoperoxidase) and anti-TCR δ antibody in red (APAAP-Fast Red) haematoxylin counterstain.

technique.[16] Double labelling was performed using an immunoperoxidase procedure followed by an immunoalkaline phosphatase method.[17] Frozen tissue sections were incubated successively with monoclonal antibody βF1 (1/1000), followed by peroxidase conjugated goat anti-mouse immunoglobulin (1/40). Both incubations were for 30 minutes. The peroxidase reaction was then developed using a substrate containing diaminobenzidine (0·6 mg/ml), H_2O_2 (0·01%), and nickel chloride (0·2 mg/ml) for five to eight minutes. Sections were then incubated with monoclonal antibody TCRδ1 (1/250) or monoclonal anti-CD3 antibody (undiluted), and the APAAP immunoalkaline phosphatase technique was then performed as detailed previously,[16 17] using either a Fast Blue or a Fast Red substrate. When the latter substrate was used sections were counterstained in haematoxylin before mounting.

To confirm the ability of the double staining technique to detect all CD3 positive cells sections from several spleens were stained by the same double labelling method using a mixture of the anti-β and anti-δ antibodies for the first reagent (developed by immunoperoxidase), followed by monoclonal anti-CD3 antibody (developed by APAAP). The same double labelling method, using monoclonal antibodies to CD8 or CD36 (undiluted and diluted 1/1000, respectively) for the first step followed by anti-β antibody, was used to show the distribution of γδ-bearing cells in relation to red pulp sinusoids.

For quantitative analysis, at least three sections of each sample were stained and three to five high power fields were counted on each slide. For each spleen the ratio of γδ to αβ cells was assessed on sections stained with the anti-δ and anti-β reagents, and also on sections stained with anti-β and anti-CD3.

Results

A total of 14 spleen samples were examined in this study. These were chosen from 16 unselected specimens on the basis of satisfactory morphological preservation and the presence of moderate numbers of CD3 positive T cells in the red pulp. Two samples in which only occasional T cells were present in the red pulp were excluded on the basis that the prevalence of a minor subpopulation of T cells would be too low for evaluation.

γδ positive cells were present in the red pulp of all samples and comprised on average 17% of all T cells (fig 1). The values for individual spleens lay in the range 6% to 22%, with the

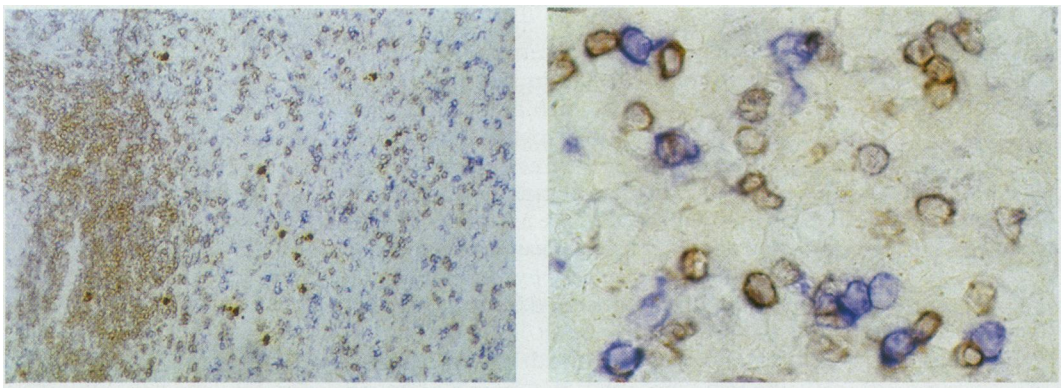

Figure 2 Double labelling of the spleen in which the highest number of γδ T cells was found (sample No 11) with anti-TCR β antibody in brown (by immunoperoxidase) and anti-TCR δ antibody in blue (APAAP-Fast Blue). Almost all cells in the T cell areas of the white pulp carry αβ TCR, whereas in the red pulp many γδ-bearing cells are seen. (No counterstain.)

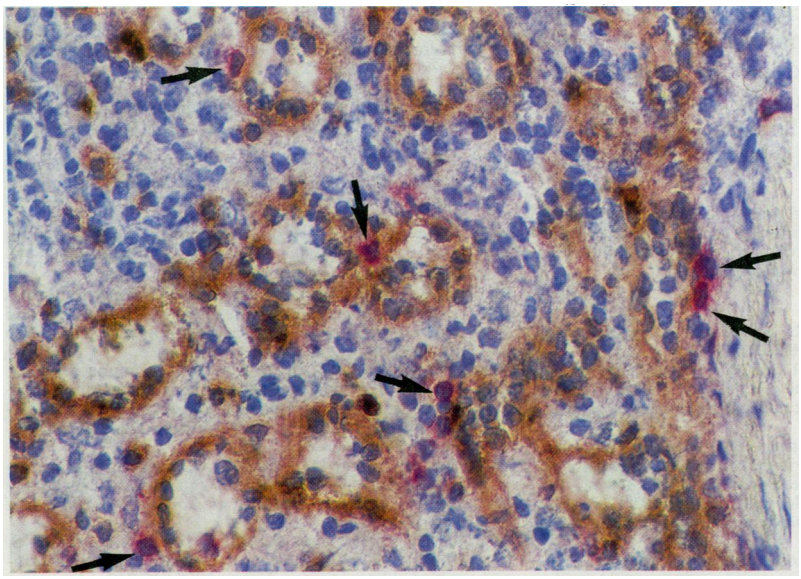

Figure 3 Double labelling of normal spleen to show the sinusoids using anti-CD36 (in brown) and γδ-bearing T cells (in red). The γδ-bearing T cells lie within the cords of the red pulp, rather than within the sinusoids, although they are often situated close to the sinusoidal endothelium. (Haematoxylin counterstain.)

exception of one sample (No 11) in which γδ-bearing cells were almost as plentiful as αβ cells (fig 2). This sample was from an African patient with an enlarged spleen in whom a lymphoma was suspected but who was subsequently found to have falciparum malaria. In all samples, including this latter case, γδ cells were scattered evenly through the red pulp with no tendency to form clusters. There was no correlation (with the exception of the case of malaria) between the percentage of γδ cells and the diagnosis or the presence of specific histological features such as increased red pulp cellularity. Double staining with antibodies to CD8 or CD36, both of which show sinusoidal lining cells, in combination with antibody to TCR δ, showed that the γδ-bearing cell population was present in the cords of the red pulp (rather than within the sinusoids) often lying close to the sinusoidal endothelium (fig 3).

The lymphoid areas of the spleen contained confluent zones of CD3 positive T cells, but, in contrast to the red pulp, most expressed TCR αβ, and only very occasional γδ cells were observed. The few T cells in B cell areas (germinal centres, mantle zones, marginal zones) were also almost all αβ-bearing cells.

The prevalence of γδ cells was also estimated in all cases by staining for β^+ cells compared with $\beta^-/CD3^+$ cells (which by implication represent the γδ-bearing T cell population). The results were closely similar to those obtained using the combination of the anti-β and anti-δ reagents, with an average value for γδ-bearing T cells of 20% (range 7–51%). When spleen sections were stained initially with a mixture of anti-β and anti-δ antibodies and then by the CD3 antibody, no CD3 positive cells that lacked β or δ reactivity were seen.

Discussion

Shortly after the γδ-bearing T cell population was recognised, immunohistological studies of the mouse suggested that this cell population was present at high numbers in gut epithelium.[18] More recently Augustin *et al* have provided evidence that 8–20% of resident pulmonary lymphocytes in the mouse are also γδ-bearing cells.[19] It has therefore been hypothesised that γδ cells might have a role in recognising foreign antigens at these sites. This distribution pattern observed in the mouse is not, however, found in human tissues. An extensive study of the distribution of γδ cells in man[15] showed no tendency for γδ T cells to be more common in any tissue other than in peripheral blood, except that in some spleen samples (out of seven adult and fetal spleen samples studied) these cells seemed to be more common in the red pulp and in marginal zones than in T cell areas, although no figures were given. More recently Bucy *et al,* by single staining of five samples using the same monoclonal anti-δ antibody as in our study, reported that γδ-bearing cells are present, "almost exclusively in splenic sinusoids", although again no figures are given for this.[14] The same group had previously shown that in chickens the equivalent of γδ T cells are also preferentially localised in the splenic red pulp where they constitute 30% of all splenic T cells.[13] In this species, however, a higher proportion of circulating T cells (about 20%) belong to this population than in man.[20]

In this study we have confirmed the observation of Bucy *et al*[14] that γδ-bearing T cells localise preferentially to the red pulp. Unlike previous authors, we estimated the prevalence of γδ-bearing cells among the total T cell population, an assessment which was greatly facilitated by the use of double immunoenzymatic techniques. This showed that δγ-bearing T cells account, in most subjects, for 6–22% of all T cells. In comparison, the average percentage in normal peripheral blood has previously been shown to be only 4% and there is hence a four- to five-fold enrichment of γδ-bearing T cells in the splenic red pulp.[15]

We did not observe increased numbers of γδ T cells in the marginal zones of the spleen as reported by Groh *et al*[15] and it was clear from double staining that the γδ T cells were found in splenic cords and not "almost exclusively in splenic sinusoids", as stated by Bucy *et al.*[14] An additional novel observation in the present study was that when spleen sections were stained initially with a mixture of anti-β and anti-δ antibodies and then by a pan-T cell CD3 antibody, no cells were detected which reacted only with the latter antibody, arguing against the existence in man of a third T cell receptor population, as has been reported in chickens by Chen *et al.*[20]

Finally, one may speculate on the clinical importance of the preferential localisation of γδ cells in the red pulp of the spleen. It has been suggested that T cells bearing the γδ receptor represent an early stage in the phylogenic and ontogenic evolution of the immune system,[21 22] and that they may be responsible for defence against highly conserved antigens.[2] Their localisation in the splenic red pulp may

therefore reflect the early development of this tissue as part of the immune system, before the appearance of organised lymphoid structures, and the high proportion of $\gamma\delta$ T cells in case 11, the patient who had malaria, may be related to their role in eliminating infectious organisms.

We are grateful to Margaret Jones for expert technical assistance. This work was supported by a grant from the Ligue Nationale de la Recherche contre le Cancer, France and by the Leukaemia Research Fund, Great Britain.

1 Brenner MB, Strominger JL, Kranger MS. The $\gamma\delta$ T cell receptor. *Adv Immunol* 1988;**43**:133–42.
2 Raulet DH. Antigens for $\gamma\delta$ T cells. *Nature* 1989;**339**:342–3.
3 Strominger JL. The $\gamma\delta$ T cell receptor and class Ib MHC-related proteins: enigmatic molecules of immune recognition. *Cell* 1989;**57**:895–8.
4 Modlin RL, Pirmez C, Hofman FM, *et al.* Lymphocyte bearing antigen specific $\gamma\delta$ T-cell receptors accumulate in human infectious disease lesions. *Nature* 1989;**339**:544–8.
5 Haregewoin A, Soman G, Hom RC, Finberg RW. Human $\gamma\delta+$ T cells respond to mycobacterial heat-shock protein. *Nature* 1989;**340**:309–12.
6 Kozbor D, Trinchieri G, Monos DS, *et al.* Human TCR-$\gamma+/\delta+$, T lymphocytes recognize tetanus toxoid in an MHC-restricted fashion. *J Exp Med* 1989;**169**:1847–51.
7 Wright A, Lee JE, Link MP, *et al.* Cytotoxic T lymphocytes specific for self tumor immunoglobulin express T cell receptor δ chain. *J Exp Med* 1989;**169**:1557–64.
8 Bluestone JA, Matis LA. TCR $\gamma\delta$ cells—minor redundant T cell subset or specialized immune system component? *J Immunol* 1989;**142**:1785–8.
9 Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. *Nature* 1988;**334**:395–402.
10 Bonneville M, Janeway CA, Ito K, *et al.* Intestinal intraepithelial lymphocytes are a distinct set of $\delta\gamma$ T cells. *Nature* 1988;**336**:479–81.
11 Goodman T, Lefrancois L. Expression of the γ-δ T cell receptor on intestinal CD8+ intraepithelial lymphocytes. *Nature* 1988;**333**:855–8.
12 Viney JP, MacDonald TT, Kilshaw PJ. T-cell receptor expression in intestinal intra-epithelial lymphocyte subpopulations of normal and athymic mice. *Immunology* 1989;**66**:583–7.
13 Bucy RP, Chen CLH, Cihak J, Losch U, Cooper MD. Avian T cells expressing $\gamma\delta$ receptors localize in the splenic sinusoids and the intestinal epithelium. *J Immunol* 1989;**141**:2200–5.
14 Bucy RP, Chen CLH, Cooper MD. Tissue localization and CD8 accessory molecule expression of T$\gamma\delta$ cells in the humans. *J Immunol* 1989;**142**:3045–9.
15 Groh V, Porcelli S, Fabbi M, *et al.* Human lymphocytes bearing T cell receptor γ/δ are phenotypically diverse and evenly distributed throughout the lymphoid system. *J Exp Med* 1989;**169**:1277–94.
16 Cordell JL, Falini B, Erber WN, *et al.* Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984;**32**:219–29.
17 Mason DY, Stein H, Naiem M, Abdulaziz Z. Immunohistological analysis of human lymphoid tissue by double immunoenzymatic labelling. *J Cancer Res Clin Oncol* 1981;**101**:13–22.
18 Takagaki Y, DeCloux A, Bonneville M, Tonegawa S. Diversity of $\delta\gamma$ T-cell receptors on murine intestinal intraepithelial lymphocytes. *Nature* 1989;**339**:712–14.
19 Augustin A, Kubo RT, Sim GK. Resident pulmonary lymphocytes expressing the γ/δ T-cell receptor. *Nature* 1989;**340**:239–41.
20 Chen CH, Sowder JT, Lahti JM, Cihak J, Losch U, Cooper MD. TCR3: A third T-cell receptor in the chicken. *Proc Natl Acad Sci USA* 1989;**86**:2351–5.
21 Campana D, Janossy G, Coustan-Smith E, *et al.* The expression of T cell receptor-associated proteins during T cell ontogeny in man. *J Immunol* 1989;**142**:57–66.
22 Janeway CA. Frontiers of the immune system. *Nature* 1988;**333**:804–6.