Clin. exp. Immunol. (1983) **53**, 672–678.

Subsets of blood, spleen and recirculating lymphocytes in man

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(Accepted for publication 11 April 1983)

SUMMARY

Lymphocyte subpopulations were characterized in human blood and spleens. In addition the spleens were perfused by a closed extracorporeal perfusion system under almost physiological conditions. Lymphocytes released from the spleen during perfusion were taken to be representative of recirculating lymphocytes. B lymphocytes were classified by their surface immunoglobulin, T lymphocytes and T lymphocyte subsets by cytochemistry, sheep red blood cell rosette formation and in some experiments by monoclonal antibodies. In the blood $71 \pm 4{\cdot}3\%$ of the lymphocytes were rosette forming cells and $23{\cdot}3 \pm 3{\cdot}8\%$ B lymphocytes. In the spleen $49{\cdot}8 \pm 3{\cdot}6\%$ were T and $53{\cdot}3 \pm 2{\cdot}1\%$ were B lymphocytes. In three spleens the mean number of $OKT3^+$ lymphocytes were $27{\cdot}6 \pm 7{\cdot}0\%$, $OKT4^+$ $8{\cdot}6 \pm 1{\cdot}4\%$ and $OKT8^+$ $13{\cdot}7 \pm 2{\cdot}2\%$. The ratio of T helper to T suppressor lymphocytes was 0·67 for the spleen and 1·7 for the blood. The lymphocytes released from the perfused spleen showed a similar distribution pattern of surface markers to that of the splenic subpopulations.

Keywords lymphocyte subsets human spleen blood lymphocyte migration

INTRODUCTION

There are now many different techniques available for classifying human lymphocytes, such as cytochemistry, rosetting techniques and immunofluorescence with monoclonal antibodies. In nearly all studies only lymphocytes from peripheral blood have been characterized in humans, although they only constitute about 2% of all lymphoid cells in the human body (Trepel, 1974). One aim of the present study was to characterize lymphocyte subpopulations in human spleens using various techniques.

The function of lymphocyte subpopulations has mainly been evaluated in *in vitro* assays. The migration kinetics of most subpopulations has not been studied *in vivo*. In Hodgkin's disease a maldistribution of T cell subsets has been described between the blood and spleen (de Sousa *et al.*, 1977; Gupta, 1980; Gupta & Good, 1981; Gupta & Tan, 1980). A reduction or increase of a subset in the blood is not necessarily indicative of a similar alteration in the whole lymphoid system, since we do not know the migratory properties of lymphocyte subclasses in health and disease. The spleen plays a predominant role in lymphocyte recirculation (Pabst & Trepel, 1975; Ford & Smith, 1979)

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and lymphocyte subpopulations differ markedly in their circulation pattern through the spleen, as shown recently for pig spleens (Binns, Pabst & Licence, 1981). Therefore, we used an established extracorporeal perfusion system (Pabst & Trepel, 1974; Reinecke & Pabst, 1980; Pabst & Reinecke, 1981) and perfused isolated human spleens. The lymphocytes released from the spleen were classified and compared to the subpopulations in the blood and splenic cell suspensions.

MATERIALS AND METHODS

Spleens. A total of 10 spleens were perfused. Nine were from patients undergoing total gastrectomy for gastric carcinoma or carninoma of the oesophagic cardia. One spleen was from a patient with idiopathic thrombocytopenic purpura. The age of the patients ranged from 45 to 76 years (mean 63·5 years). The splenic weight ranged from 70 to 210 g (mean 152 g).

Perfusion. Spleens were kept sterile after splenectomy and carried to the laboratory in cold 0·9% sodium chloride solution immediately after removal. In the laboratory they were flushed with Collin's solution (Fresenius, Bad Homburg, West Germany) and cooled to 4°C. A part of the spleen, not flushed by saline, was removed for cell suspension. The spleen was then connected to the perfusion system as described earlier (Pabst & Reinecke, 1981). In brief, this closed-circuit system consisted of a roller pump, oxygenator, perfusion chamber for the spleen and a regulating system to keep the pH and temperature constant. The perfusate consisted of 90% RPMI 1640 medium with 10% pooled human serum, supplemented with glucose to a final concentration of 300 mg % and 35 g/l dextran (60–90,000 mol. wt, Serva, Heidelberg, West Germany). During perfusion the temperature, flow, arterial pressure, arterial and venous pO_2, pCO_2 and pH were gradually brought up to a constant level. Then the spleens were perfused at 37°C for at least 1 h, which is long enough to reach a balance between emigrating and immigrating lymphocytes (Pabst & Trepel, 1975; Binns *et al.*, 1981).

Cell preparation. Venous blood was taken from each patient just before splenectomy or before an intra-operative blood transfusion. Lymphocytes were separated by centrifugation on a Ficoll density gradient. Cell suspensions of splenic tissue were made by teasing pieces of unflushed spleen. Lymphocytes from washing fluid and from perfusate were concentrated by centrifugation. Before carrying out the surface marker studies, viability was tested by the trypan blue dye exclusion test, which in each case resulted in $>90\%$ viable cells.

Cell surface markers. Sheep red blood cell rosettes were attained according to the method described by Kaplan & Clark (1974), using AET sensitized SRBC. Surface Ig was detected by direct immunofluorescence using FITC coupled $F(ab)_2$ rabbit anti-human Ig antisera (Behringwerke, Marburg, West Germany). T cell subsets were evaluated using the indirect immunofluorescence technique with monoclonal antibodies OKT3, OKT4 and OKT8 (Ortho, Raritan, New Jersey, USA) and FITC coupled goat anti-mouse IgG antisera (Cappel, Cochranville, Pennsylvania, USA). At least 200 cells were counted on two different slides for each compartment under fluorescence microscopy. Null cells were calculated by subtracting E rosette percentages and surface immunoglobulin (sIg) percentages from 100%.

ANAE staining. A slightly modified method of cytochemistry for α-naphthyl acetate esterase as described by Horwitz *et al.* (1977) and Müller *et al.* (1981) was carried out. In brief, smears were fixed in cold buffered formalin acetone for 30 s, rinsed in distilled water and incubated in phosphate buffer with a pH of 5·8 for 2 h at 37°C, with α-naphthyl acetate as substrate and pararosanilin as the coupling agent. The slides were counterstained with methyl green. Lymphocytes with 1–4 distinct dots produced by the ANAE reaction were considered T cells.

Statistical analyses. The Student's *t*-test was used. The data are given as the mean ± standard error of the mean. Statistical significance was defined as $P<0{\cdot}05$.

RESULTS

Histological sections of the spleen after 1–2 h perfusion showed no obvious cell damage or organ

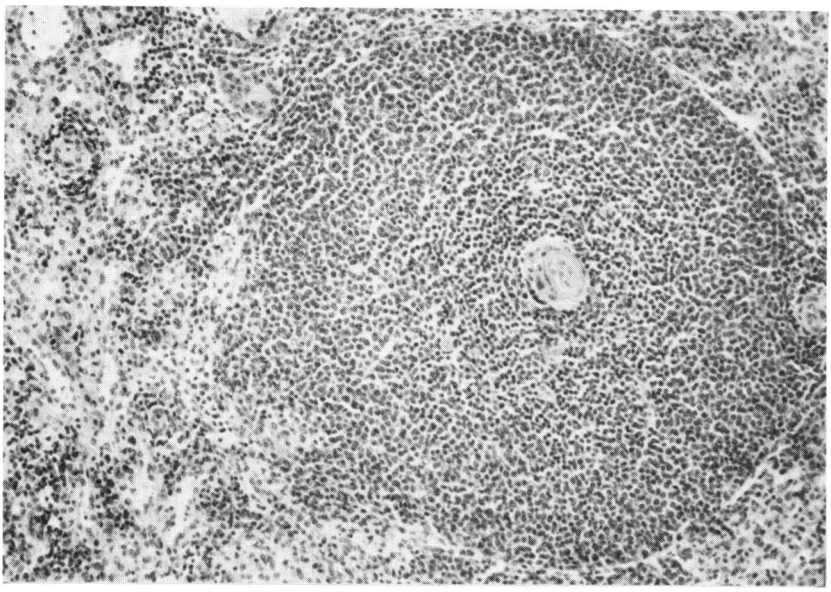

Fig. 1. A histological section of a human spleen after *ex vivo* normothermic perfusion demonstrates well preserved parts of the red and white pulp. (2 μm, Giemsa, $\times$ 100).

destruction. The typical architecture of the spleen was well preserved (Fig. 1). The results of all 10 perfused spleens and conventional markers are shown in Fig. 2. T cell percentages decreased gradually from the blood to the perfusate. The blood, compared to all other compartments with regard to T cell percentages, showed statistically significant differences. B cell percentages, conversely, increased from blood via washing fluid and spleen to perfusate. Comparison of the compartments showed significant differences in blood to spleen, blood to perfusate, washing fluid to spleen and washing fluid to perfusate. Null cells were highest in the washing fluid and significantly different compared to all other compartments. They were lowest in spleen cell suspensions. Comparison of the spleen and perfusate showed no significant differences in any lymphocyte subpopulation percentages.

The results of three spleens, to which all marker techniques were applied, are summarized in Fig. 3. With regard to T cells, decreasing percentages were noted from blood to spleen, whereas an increase was seen in the perfusate. Comparing blood to the other compartments, calculated on the

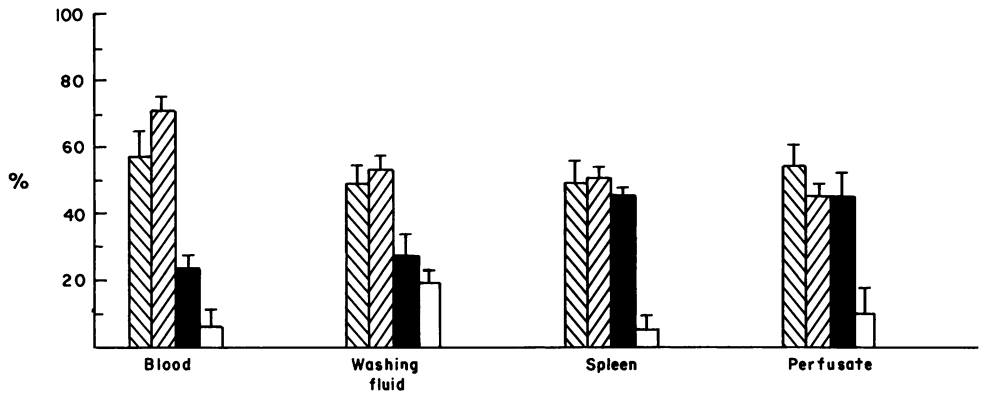

Fig. 2. Mean values $\pm$ standard error of the mean for lymphocyte subpopulations in different compartments in 10 experiments. □ = Null cells = (100% − [E rosettes % + sIg%]); ■ = sIg^+; ■ = $ANAE^+$; ■ = E rosettes.

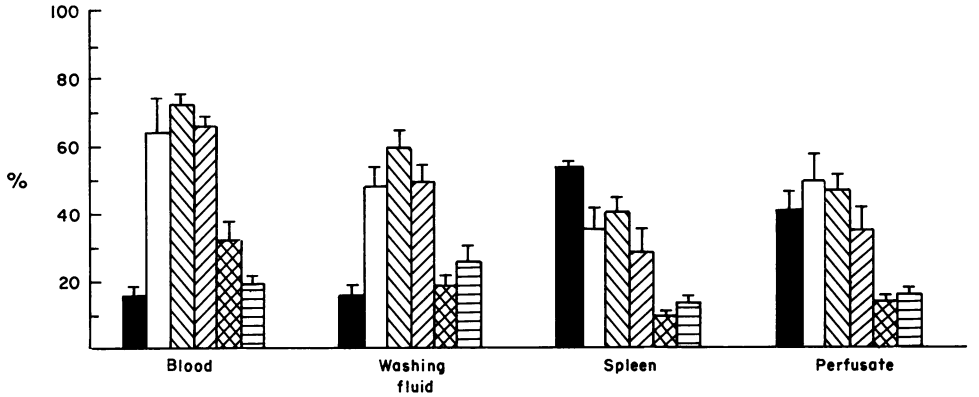

Fig. 3. Mean proportion of B and T lymphocytes and T lymphocyte subsets in different compartments in three experiments. ■ = sIg^+; □ = $ANAE^+$; ▩ = E rosettes; ■ = $T4^+$; ■ = $T4^+$; ■ = $T8^+$

basis of E^+ and $OKT3^+$ separately, all differences showed statistical significance. The same was true comparing washing fluid to spleen, but spleen to perfusate and washing fluid to perfusate were not significant. Again B cells behaved conversely. They increased from blood through washing fluid to spleen, but decreased again in the perfusate. All differences except blood to washing fluid and spleen to perfusate were significant. Null cells were highest in washing fluid and lowest in the spleen, thus showing the same behaviour as noted for all 10 spleens. $OKT3^+$ cells showed the same tendencies as E^+ cells. However, percentages were lower than those of E^+ in all compartments examined. Looking at T helper and T suppressor cells, defined as $OKT4^+$ and $OKT8^+$ respectively, the results in the blood were as expected. Surprisingly, in all other compartments examined $OKT8^+$ cells rose above $OKT4^+$ cells. The lowest OKT4/OKT8 ratio was observed in the spleen with $0{\cdot}67 \pm 0{\cdot}2$. T helper cells differed significantly in the compartments compared, except washing fluid compared to perfusate. T suppressor cell percentages differed significantly only when blood to spleen and blood to perfusate were compared. All other possible combinations showed no significance.

DISCUSSION

Organ preservation during extracorporeal, normothermic, asanguinous perfusion of pig spleens and human spleens is well documented (Pabst & Trepel, 1974; Reinecke & Pabst, 1980). The relevance of this technique for the *in vivo* situation has been shown in cell cycle studies (Pabst & Trepel, 1976), as well as in migration studies in the pig (Pabst & Trepel, 1975; Binns *et al.*, 1981). This system has also been used for studies on the lymphocyte production in human spleens (Pabst & Reinecke, 1981).

There is little data on the distribution of T and B lymphocytes and their subsets in healthy man, except for a number of studies concerning peripheral blood lymphocytes. For example, Raeman *et al.* (1981) analysed peripheral blood lymphocytes in 21 healthy people and found 73–85% E rosette forming cells, 2–12% sIg^+ cells, 76–86% $OKT3^+$ cells, 35–55 $OKT4^+$ cells and 18–32% $OKT3^+$ cells. Reinherz *et al.* (1981), using FACS analysis of peripheral blood lymphocytes, found $67 \pm 3\%$ $OKT3^+$, $41 \pm 2\%$ $OKT4^+$, $20 \pm 1\%$ $OKT8^+$ and $10 \pm 1\%$ Ia^+ cells (corresponding to B cells). Data of other groups are similar (Vanderbecken *et al.*, 1982; Mascart-Lemone *et al.*, 1982; Kornfeld *et al.*, 1982). The corresponding values for peripheral blood lymphocytes in this study lie in the same range: $71 \pm 4{\cdot}3\%$ (mean ± s.e.) rosette forming cells, $23{\cdot}3 \pm 3{\cdot}8\%$ sIg^+ cells, $65{\cdot}4 \pm 2{\cdot}8\%$ $OKT3^+$ cells, $36{\cdot}9 \pm 6\%$ $OKT4^+$ and $22 \pm 2{\cdot}6\%$ $OKT8^+$ cells.

To find comparable data on human spleen cell subpopulations is more difficult than for blood. In Table 1 the few published data on so called 'normal control spleens' are summarized for comparison. The very low helper cell/suppressor cell ratio of 0·67 might be a sign of imbalance due

Table 1. Chronological summary of the relative number of lymphocyte subpopulations for 'normal' human spleens from the literature, in comparison to data from the present study. (SRBC = sheep red blood cells, EAC rosettes = red cells coated with antibody plus complement, MoAb = monoclonal antibody; sIg = surface Ig by direct immune fluorescence; T_H = T helper cells, T_S = T suppressor lymphocytes).

Number of spleens	Source of splenic tissue	Method	Lymphocyte subsets (%) B	Lymphocyte subsets (%) T	Author
12	Gastric cancer, splenic cyst, pancreatitis	SRBC rosetes, EC rosettes	58	26·3	Visakorpi & Repo (1973)
5	Idiopathic splenomegaly, ITP, hiatus hernia, autoimmune haemolytic anaemia	PHA transformation, sIg	57	28	Kauer *et al.* (1974)
7	Traumatic ruptures, intraabdominal malignancies, portal hypertension, haemorrhagic cyst	SRBC rosettes, sIg	45	36·5	Habeshaw & Stewart (1974)
3	Carcinoma of the lung, esophagus and brain	SRBC rosettes, EAC rosettes	18·2	75·8	de Sousa *et al.* (1977)
11	Spleen aspirates 'shortly' after cardiac arrest	SRBC rosettes, sIg	21	44	Jønssen & Christensen (1977)
5	Unclear origin	SRBC rosettes, sIg	45	35	Gupta & Tan (1980)
5	Traumatic rupture	SRBC rosettes, sIg	49·5	39·4	Rocha *et al.* (1982)
Not given	Traumatic rupture	Fe receptors for IgM, IgG	Not given	20–50 T_H T_S 5–20 25–65	Gupta (1980)
2	Wegeners granuloma, suspected lymphoma, not confirmed	SRBC rosettes, anti-HLA-DR OKT MoAb	13	61 49 T_H T_S 35 27	Dorreen *et al.* (1982)
10	Gastric cancer, ITP	ANAE, SRBC rosettes, sIg	45	49·8	This study
3		OKT MoAb, sIg	53·3	27·6 T_H T_S 8·6 13·7	

to underlying disease, i.e. gastric carcinoma, as observed for other diseases (Gupta & Good, 1981), or an influence of age (Mascart-Lemone *et al.*, 1982) or both. However, in the study by Gupta (1980), the T_H/T_S ratio was also less than 1 for normal spleens.

The main purpose of these experiments was to show the distribution of lymphocyte subpopulations in the different compartments. In the young pig, with a high number of null cells in the blood, the blood lymphocyte composition differs markedly from that of lymphocytes released from the perfused spleen (Binns *et al.*, 1981). In this animal it is interesting that there is a decrease to almost zero in the null cells in the perfusate with a corresponding increase in B and T cell percentages. In the present study, differences are not as clear as in the pig. Yet there is a distinctly different composition of blood, spleen and perfusate. Obviously the splenic washing fluid most resembles the peripheral blood, indicating that the origin of these lymphocytes is the intravascular space. On the other hand, the perfusate composition was comparable to the splenic cell suspension, but only partly resembles the blood composition. The splenic lymphocytes can be subdivided into two different pools. One can be rapidly mobilized and resembles the blood composition, while the other can only be slowly mobilised and is more comparable to splenic lymphocytes (Pabst & Trepel, 1975). Indeed, in the statistical analysis blood differs significantly compared to spleen and perfusate,

whichever marker is used (except for ANAE staining). The same is true comparing washing fluid to spleen, whereas spleen compared to perfusate only differs significantly in $OKT4^+$ cells.

The human blood, studied so often, only reflects about 2% of lymphocytes in the body as a whole, while the spleen itself consists of 15% of the total number of lymphocytes (Trepel, 1974). Therefore, changes in the blood composition of lymphocyte subgroups say little about their distribution on the whole. However, there are diseases such as Hodgkin's disease, in which a maldistribution of lymphocyte subsets between the blood and spleen has been described. Splenectomy in these patients resulted in a normalization of the lymphocyte subsets in the blood (Gupta & Tan, 1980).

This study was supported by the Deutsche Forschungsgemeinschaft (Re 560/1-1). We wish to thank Professor Pichlmayr, Department of Surgery, and his colleagues who let us study the spleens. We are grateful to M. Kaatz, M. Reis and H. Wilting for their technical help and to Sheila Fryk for correcting the English of the manuscript.

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