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Distribution of Different Cell Types in the Lymphoid Organs of the Mouse, as Determined with Sera against Thymus and Peyer's Patches

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Summary. Anti-thymus serum (ATS) absorbed with Peyer's patch cells and anti-Peyer's patch serum (APS) absorbed with thymus cells were prepared. ATS treatment of mice resulted in a suppression of the immune response, measured with *Vibrio cholerae* as an antigen. APS did not have this effect. ATS and APS were used in the indirect fluorescence test to detect T and B_p cells. The cells staining with APS were called B_p cells to indicate that APS might only detect a special fraction of B cells (mature B cells). In Peyer's patches 66 per cent of the cells reacted with APS (B_p cells), whereas only 19 per cent cells reacted with ATS (T cells). The percentage of T cells in the lymph nodes was high (73 per cent), whereas only 14 per cent B_p cells were found in this organ. In the spleen almost equal numbers of B_p and T lymphocytes (32 per cent and 33 per cent) were detected. However, 35 per cent of the lymphocytes were non-reactive with either anti-serum. Bone marrow contained only small numbers of reactive cells (1 per cent T and 2 per cent B_p cells).

Treatment of mice with dimethylbenzanthracene (DMBA) caused an increase in the number of non-reactive cells in Peyer's patches and a dramatic decrease in the number of B_p cells. The relative increase of the number of T cells in spleen and lymph nodes was not found in Peyer's patches. Cortisone acetate seems also to act primarily on B cells, especially on those of Peyer's patches. In this case also an increase in the proportion of T cells in the spleen was detected.

INTRODUCTION

Reif and Allen (1964, 1966) were the first to find the θ (theta) antigen in lymphatic and brain tissue of mice. They suggested that for lymphocytes θ antigen was only present on thymus-dependent cells. This was confirmed by Raff (1969) and Schlesinger and Yron (1969a and b). B cells are distinguished either by their property of carrying surface immunoglobulin (Ig) (Raff, Sternberg and Taylor, 1970) or a cell surface antigen, called MBLA (Raff, Nase and Mitchison, 1971).

Reconstitution experiments with irradiated mice receiving lymphoid cells from various organs have shown that cells from Peyer's patches together with cells from thymus or lymph nodes re-established the immune response, suggesting that B cells predominated

in Peyer's patches (Veldkamp, Gaag and Willers, 1973). Anti-Peyer's patch serum (APS) was prepared in order to study the distribution of cells with a specific B-cell antigen in the lymphoid organs of the mouse. In comparison a rabbit anti-mouse thymus serum (ATS) was used to detect T cells. Raff's indirect fluorescence technique (1970) on living cells was used. These experiments were also done on animals which had been treated with immune suppressive agents, in order to determine the shift in relative amounts of B_p and T cells in the lymphoid organs after such treatment.

MATERIALS AND METHODS

Animals

Male Swiss and N inbred mice, 12 weeks of age, were purchased from the Central Institute for the Breeding of Laboratory Animals, TNO (Zeist, The Netherlands).

F_1 (DBA × C57Bl) mice were thymectomized at the age of 7 weeks and lethally irradiated 1 month later. They were immediately reconstituted with bone marrow cells treated with anti-θ serum and complement. These mice and the untreated control F_1 mice were a gift from Dr W. van Muiswinkel, Department of Cell Biology, Erasmus University, Rotterdam, The Netherlands.

Cell suspensions

Cell suspensions from the lymphoid organs of the mice were prepared as described by Kerckhaert, Benner and Willers (1973). The spleen suspensions were treated with Tris-buffered NH_4Cl to lyse the RBC and passed through a short column of glass wool (Raff, 1970).

Antisera

Anti-thymocyte serum (ATS) and anti-Peyer's patch cell serum (APS) were prepared in New Zealand White rabbits. The animals received two intravenous injections of 2×10^8 cells from Swiss mice with an interval of 2 weeks. Ten days after the last injection the animals were bled. IgG was isolated from APS and ATS according to Stanworth (1960). Portions of 1 ml of the IgG solutions were absorbed twice with 2×10^8 Swiss Peyer's patch cells for the ATS and with 10^9 Swiss thymus cells for the APS preparation. For the staining of the F_1(DBA × C57Bl) organs the ATS and APS were absorbed with the appropriate organs of these mice. The absence of antibodies against mouse serum proteins in ATS and APS was verified by gel immunoelectrophoresis.

Immunofluorescent staining

Indirect immunofluorescence was performed as described by Raff (1970), except for the following modifications. A fluorescent goat anti-rabbit-IgG serum (Nordic, Tilburg, The Netherlands) was used throughout. With each type of lymphoid tissue used in these experiments, the ATS and APS was tested in various dilutions to be certain that the percentage of cells staining reached a plateau at high concentrations of the antisera. The percentage of fluorescent cells was the same when ATS was used undiluted or diluted 1 : 5 or 1 : 10. However, the fluorescence was brighter undiluted or 1 : 5. For APS the percentage of fluorescent cells was the same undiluted or 1 : 5, but in the dilution 1 : 10 the percentage fluorescent cells decreased. The brightness of the fluorescence was better when APS was used undiluted. Thus in further experiments ATS was used in a dilution

of 1 : 5, whereas APS was used undiluted. Cells incubated with normal rabbit serum and with Eagle's medium were used as controls. The specificity of APS was tested on thymus cells. It was not possible to test the specificity of ATS because no lymphoid organ contains only B cells. A Leitz Orthoplan fluorescence microscope with a Ploem illuminator was used. Cells positive with ATS are called T cells. Those positive with APS are called B_p cells, because they presumably belong to the B series but may carry an antigen present on cells of Peyer's patches and not on all B cells.

Immunosuppressive treatment

N mice were injected intraperitoneally with 3 mg cortisone acetate (Organon, Oss, The Netherlands). Three days later the cells of spleen, lymph nodes and Peyer's patches were used for immunofluorescence. Dimethylbenzanthracene (DMBA, Fluka, A.G. Switzerland) was suspended in corn oil. 1·5 mg DMBA in 0·3 ml corn oil (Nutritional Biochemicals Corporation, Cleveland, Ohio) was injected intramuscularly. After 7 days the lymphocytes of spleen, lymph nodes and Peyer's patches were used for immunofluorescence with ATS and APS.

RESULTS

INFLUENCE OF ATS AND APS TREATMENT OF MICE ON THE IMMUNE RESPONSE

Four groups of eight Swiss mice each received intraperitoneally 0·4 ml of either ATS, APS, normal rabbit serum (NRS) or saline on 17 hours before and 1 and 23 hours after immunization with 10^9 killed *V. cholerae* bacteria. The number of plaque-forming cells (pfc) was determined 7 days after immunization (Veldkamp *et al.*, 1973). Fig. 1 shows that APS has the same suppressive effect as NRS. On the other hand ATS has a significantly ($P = <0{\cdot}01$) stronger suppressive activity than both the other sera.

PERCENTAGES OF B AND T LYMPHOCYTES

Living cell suspensions of lymphoid organs of N mice were treated with ATS and APS and stained with fluorescent goat anti-rabbit IgG serum. The numbers of fluorescent

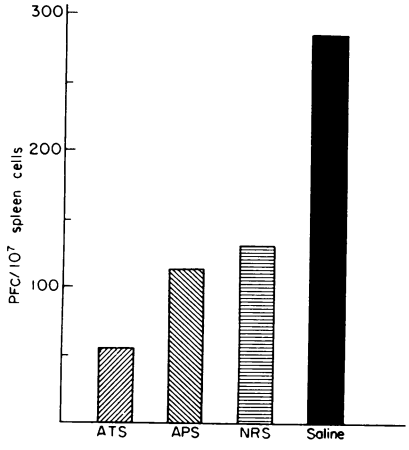

FIG. 1. Influence of ATS and APS treatment of mice on the immune response against *Vibrio cholerae*.

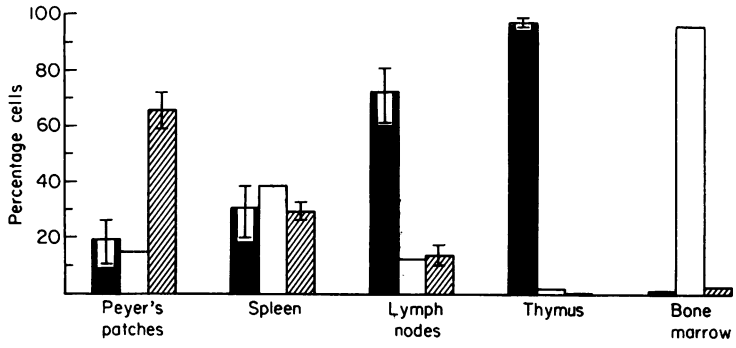

FIG. 2. Percentages of B_P and T lymphocytes in different lymphoid organs of mice determined with indirect fluorescence on living cells with (■) ATS and (▨) APS. (□) Percentage of cells not staining.

cells were counted (Fig. 2). No thymus cells stained with APS, but almost all thymus cells were positive with ATS. Peyer's patch cells included a high percentage (66 per cent) positive with APS, whereas ATS reacted with 19 per cent of these cells. A high percentage of ATS positive cells (73 per cent) was found in the lymph nodes. Only 14 per cent of the cells of this organ reacted with APS.

In the spleen APS and ATS positive cells were present in about equal numbers (32 and 33 per cent). In bone marrow only very small numbers of APS and ATS positive cells could be detected. Most of the cells in the bone marrow (97 per cent) did not react with either serum. The percentages of non-reacting cells for Peyer's patches, spleen and lymph nodes were 15, 35 and 13.

In additive experiments ATS and APS were used together. From Table 1 it can be seen that the percentage of cells staining with ATS and APS combined is about equal to the sum of the percentages of cells staining with ATS or APS separately.

TABLE 1

STAINING OF CELLS OF SPLEEN, LYMPH NODES AND PEYER'S PATCHES WITH ATS AND APS SEPARATELY AND COMBINED

Cell source	Percentage of cells giving immunofluorescence with		
	ATS	APS	ATS+APS
Spleen	35	34	70
Peripheral lymph nodes	77	16	98
Peyer's patches	21	61	87

The percentages are the mean of those of three different experiments.

THYMECTOMIZED MICE

The results of indirect fluorescence with F_1 (DBA × C57Bl) mice thymectomized as adults, lethally irradiated and reconstituted with syngeneic bone marrow, and normal mice are outlined in Table 2. Thymectomized mice show a marked decrease in cells staining with ATS. A relative high percentage of cells in lymph nodes stains with APS. The percentage of APS positive cells in Peyer's patches of thymectomized mice was far lower than in normal animals, while that of the spleen was unchanged.

NUMBER OF B AND T CELLS AFTER IMMUNOSUPPRESSIVE TREATMENT

A number of N mice were treated either with DMBA or with cortisone acetate. At least six preparations of pooled cells of lymphoid organs of four animals were stained for B_p and T cells (Table 3). In DMBA-treated animals the relative number of B_p cells is impaired in spleen, lymph nodes and Peyer's patches. In the latter organ the number of non-reactive cells was increased from 15 per cent for normal animals to 81 per cent for DMBA-treated animals. In spleen and lymph nodes there is a relative increase in the number of ATS positive cells; in the spleen this occurs at the cost of non-reactive cells and without material reduction of B_p cells. Treatment with cortisone acetate caused also an increase in the number of ATS positive cells in spleen and Peyer's patches. In the latter organ the number of APS positive cells is reduced from 66 per cent for normal

TABLE 2

RESULT OF INDIRECT IMMUNOFLUORESCENCE WITH CELLS OF THYMECTOMIZED AND NON-THYMECTOMIZED (DBA × C57Bl) F_1 MICE

Treatment of mice	Cell source	Percentage of cells staining with		
		ATS	APS	Negative
None (normal mice)	Spleen	37	48	15
Th x*	Spleen	1	50	49
None	Lymph nodes	90	10	0
Th x	Lymph nodes	4	72	24
None	Peyer's patches	25	65	10
Th x	Peyer's patches	1	10	89

*Th x = adult thymectomized, lethally irradiated and reconstituted with syngeneic bone marrow treated with anti-θ serum and complement.

TABLE 3

INDIRECT IMMUNOFLUORESCENCE WITH ATS OR APS IN LYMPHOID CELL SUSPENSIONS OF NORMAL AND IMMUNOSUPPRESSED MICE

Organ	Untreated		DMBA (1½ mg)		Cortisone (3 mg)	
	ATS (%)	APS (%)	ATS (%)	APS (%)	ATS (%)	APS (%)
Spleen	33	32	53	28	70	34
Lymph nodes	73	14	86	6	88	9
Peyer's patches	19	66	10	9	44	18

At least six preparations of pooled cells of lymphoid organs of four animals were stained for B_p and T cells.

mice to 18 per cent for cortisone-treated mice. The number of non-reactive cells is increased to 38 per cent. The non-reactive cells are no longer detectable in the spleen.

DISCUSSION

A search for the mammalian equivalent of the avian bursa has focused on the bowel and the organized collections of intestinal lymphoid tissue known as Peyer's patches. Extirpation experiments of this tissue in rabbits have lent some support to this hypothesis

(Perey, Frommel, Hong and Good, 1970). Our observations suggest that Peyer's patches lack the properties of a secondary lymphoid organ and are a good source of precursors of antibody-forming cells. These cells seem to be more mature than bone marrow cells (Veldkamp *et al.*, 1973).

For the preparation of anti-sera to detect B cells several methods were used. Raff, Nase and Mitchison (1971) used foetal liver cells which had matured in the spleens of irradiated mice to prepare anti-MBLA serum. Rabellino, Colon, Gray and Unanue (1971) used an anti-mouse Ig serum for the detection of B cells which are supposed to carry receptors of immunoglobulin nature. Potworowski (1972) prepared an anti-bursa serum to detect B cells in chickens. On the basis of previous results (Veldkamp *et al.*, 1973) we decided to prepare an antiserum against Peyer's patch cells (APS) in order to study the presence of a common antigen on these cells (B_p cells or mature B cells) and corresponding cells in the other lymphoid organs. This serum and the ATS were prepared with cells from Swiss mice and used in experiments on cells of N mice or F_1 (DBA × C57Bl) mice to exclude reactions with transplantation antigens. Our results with immunofluorescence and heterologous ATS are in accordance with the results of Raff and Owen (1971) with anti-θ serum and cytotoxicity and with the immunofluorescence studies of Raff *et al.* (1970). The absence of B cells in the thymus was demonstrated by Raff *et al.* (1971) using anti-MBLA serum, by Rabbelino *et al.* (1971) with anti-Ig serum and with our APS. Potworowski (1972) found in the chicken thymus as well as 50 per cent T cells 7 per cent cells reactive with anti-bursa serum. The number of B_p cells in Peyer's patches (66 per cent) found with APS corresponds with the number found by Raff *et al.* (1971) with anti-MBLA serum. The bursa however contained only 40 per cent cells reactive with anti-bursa serum (Potworowski, 1972). The spleens showed slight differences in the B-cell content, when tested with APS (32 per cent), anti-MBLA (56 per cent) and anti-Ig (49 per cent). Differences are also found in the lymph nodes: APS (14 per cent), anti-MBLA (28 per cent) and anti-Ig (7 per cent). The greatest differences, however, are found in the bone marrow, where with APS (2 per cent), anti-MBLA (39 per cent) and anti-Ig (9 per cent) positive cells are found. In spleen, lymph nodes and bone marrow the numbers of positive cells are highest when anti-MBLA serum is used, indicating that probably also immature B cells are detected with anti-MBLA serum. Anti-bursa serum gives for the chicken-spleen the same number of B cells (Potworowski, 1972) as is found with APS in the mouse spleen. The number of cells not reacting with either serum was also about the same in the mouse and in the chicken spleen. These non-reactive cells are present in highest numbers in bone marrow of the mouse. This indicates that most of the bone marrow cells are immature and do not carry the antigens for T or mature B cells.

Peyer's patches also contain about 19 per cent T cells. This is in accordance with the results of Potworowski (1972) who found 10 per cent T cells in the bursa. This cannot be taken as indicating that the bursa and Peyer's patches are secondary lymphoid organs. Although T cells are present in Peyer's patches, reconstitution experiments show that these cells do not act as helper cells and need thymus cells in addition to give an immune response against a thymus-dependent antigen (Veldkamp *et al.*, 1973). The experiments with the thymectomized mice show that while the percentage of ATS positive cells in these mice is reduced considerably, a high increase in the percentage of APS positive cells was found in the lymph nodes and no change in the spleen. However, Peyer's patches showed a greatly reduced percentage of APS positive cells. This could be due either to a preferential homing of the infused bone marrow cells in spleen and lymph nodes or to a migration of

bone marrow cells (originally containing only 2 per cent B_p cells), after maturation in Peyer's patches, to the partly empty spleen and lymph nodes. This suggests that immature B cells remain in Peyer's patches.

Treatment of mice with DMBA confirms the suggestion that DMBA acts mainly on B cells (Rowland and Hurd, 1970). The greatest difference is found in the composition of Peyer's patches. The increase of the number of non-reacting cells might be caused by an influx of bone marrow cells. The relative increase of T cells in spleen and lymph nodes was not detected in Peyer's patches. Cortisone acetate seems also to act primarily on B_p cells, especially on those of Peyer's patches. The increase of the number of T cells in the spleen and Peyer's patches can be explained by migration of T cells from the thymus to these organs. Several authors (Blomgren and Andersson, 1971; Cohen, Fishbach and Claman, 1970) found that after cortisone treatment the number of cells in the thymus was reduced with 95 per cent. In the spleen all non-reacting cells seem to be replaced by T cells. This is in accordance with the results of Snippe, Bruynzeel and Willers (1973) who found that spleens of cortisone-treated N mice were far more active in the mixed lymphocyte reaction than spleen cells of normal mice. It is not known whether the non-reacting cells of the spleen have been killed by cortisone treatment or have migrated to other organs.

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