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Precursors of B-lymphocytes with surface IgM and their possible migration from bone marrow to peripheral lymphatic tissues*

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Summary

The lymphocytes with surface immunoglobulins (sIg) represent precursors of B-lymphocytes and were studied in ontogeny of pigs by autoradiography using iodinated monospecific anti-μ, anti-γ or anti-α antibodies. The earliest detection of $sIgM^+$ small lymphocytes was at 44 days of gestation in liver, followed at 51 days in spleen and at 60 days in bone marrow, the thymocytes being sIg-negative. The first circulating small lymphocytes were detected at 38–40 days and are most probably thymus-derived.

In adult pigs as well as after immunization of germ-free piglets IgM remains to represent the surface Ig on bone marrow lymphocytes while in peripheral lymphatic tissues there is appearance of lymphocytes labelled with anti-γ or anti-α. The percentage of $sIgM^+$ lymphocytes in bone marrow is extremely small and also the density of sIgM per cell is low.

The model of foetuses and newborn precolostral piglets has proved useful for studies of generation of B-lymphocytes with surface immunoglobulin (sIg) determinants because the six layer placental barrier prevents transfer of antigens and maternal immunoglobulins. In our earlier studies we demonstrated that surface Ig molecules which serve for primary recognition of the antigen on foetal B-lymphocytes in pigs are of the IgM class (*Jarošková et al.*, 1973). The present paper deals with the expression of sIg on lymphocytes from various organs of non-stimulated and immunized piglets and pigs. Bone-marrow-derived lymphocytes were determined by autoradiography using iodinated monospecific anti-μ, anti-γ or anti-α antibodies and simultaneously the thymus-derived lymphocytes were determined by the spontaneous E-rosette test with SRBC.

No sIg^+ cells were detected in cell suspensions from yolk sacs tested in foetuses aged 18 to 25 days of gestation at which time there is haemopoietic activity in this organ.

The first IgM-bearing lymphocytes were found in the foetal liver on the 44th day, in the spleen on the 51st day and in the bone marrow on the 60th day, the thymocytes being sIg^- Towards the end of gestation the lymphopoietic activity of the foetal liver and the number of $sIgM^+$cells decreases and at the same time there is an increase in the number of $sIgM^+$cells in the bone marrow (Fig. 1). The foetal liver was also the first site where IgM-synthesizing cells were demonstrated on the 38th day of gestation (*Prokešová et al.*, in press), i. e. simultaneously with the earliest morphological classification of small lymphocytes. Thus the foetal liver is the first site of generation of B-lymphocytes in pigs, similarly as in other mammals (*Gathings et al.*, 1976). However, we did not demonstrate in foetal pig liver an antigen-independent IgM-IgG switch in the expression of surface Ig, which was described to occur in the bursa of Fabricii in chickens (*Kincade* et al., 1971).

The foetal spleen might be another site of generation of B-cells beginning the 51st day of gestation. However, the majority of IgM-positive splenocytes show a stronger labeling with anti-μ than liver or bone marrow lymphocytes suggesting that the newly formed $sIgM^+$ cells rapidly mature under microenvironmental conditions of the spleen and acquire an increased density of IgM receptors (Fig. 1, Fig. 2).

In all the organs tested the majority of lymphocytes with sIgM in foetuses and non-immunized precolostral piglets were morpho-

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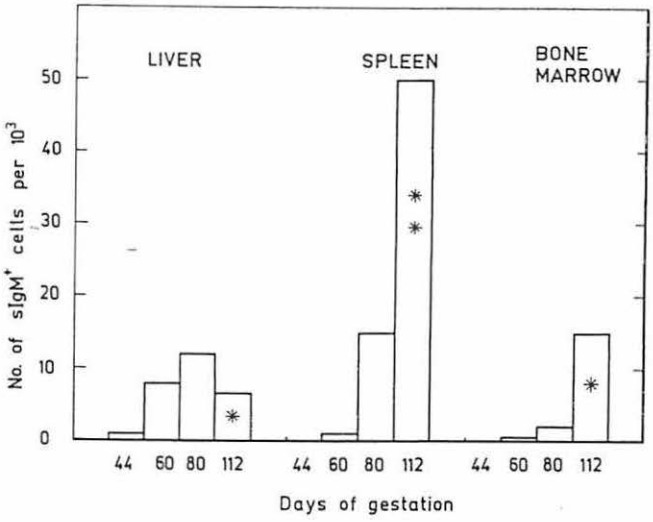

Fig. 1 The number of lymphocytes labelled with ^{125}I-anti-μ. No lymphocytes were found to be labelled with anti-γ or anti-α.
The average number of ^{125}I-grains per lymphocyte: 6.8 (*) and 25.6 (**).

logically described as small lymphocytes (Fig. 2a). Large sIg$^+$ activated lymphocytes and blast-like cells were found in substantial numbers in spleen and lymph nodes (not in bone marrow) only after immunization of germ-free (GF) piglets or adult pigs. However, in the mouse, large IgM-bearing cells were described to be major constitutent of neonatal spleens and to represent predecessors of IgD-bearing cells (*Goodman et al.*, 1977).

After immunization of GF piglets with HSA in Freund adjuvant we demonstrated a significant rise in the number of sIgM$^+$ lymphocytes in peripheral lymphatic tissues (spleen and lymph nodes), which may be due to an enhanced immigration of precursor B-cells from the bone marrow as well as to clonal expansion of sIgM$^+$ cells *in situ*. In the spleen there was also appearance of substantial numbers of cells which bind anti-γ and in mesenteric lymph nodes also of cells which bind anti-α (Table I). In contrast, in bone marrow IgM continues to represent the surface Ig even after immunization and the majority of sIgM$^+$ lymphocytes show weak labelling. We may conclude that the putative sIgM-sIgG or sIgM-sIgA switch does not occur in bone marrow. If such a switch occurs at all, it takes place only after antigenic stimulation and only in peripheral lymphatic organs. However, as judged by the labelling pattern,

Table I

Percentage of sIg$^+$ lymphocytes in GF control piglets and GF piglets after immunization with HSA-FA (10 days)

	Mesenteric lymph nodes		
	anti-μ	anti-α	anti-γ
Control	4	<0.1	<0.1
HSA-FA	11	12	2
	Bone marrow		
	anti-μ	anti-α	anti-γ
Control	2	<0.1	<0.1
HSA-FA	2	<0.1	<0.1

the binding of anti-γ or anti-α to lymphocytes need not occur via the membrane-bound IgG or IgA receptor molecules, but may be due to cytophilic adherence of labile IgG from the environment of highly activated, antibody-secretory cells in peripheral lymphatic organs (Fig. 2).

Although immunoglobulins of IgG and IgA class cannot be demonstrated by surface Ig labelling on lymphocytes of precolostral non-immunized piglets, lymphoid cells which have the potentiality to synthesize IgG or

Fig. 2a A lymphocyte labelled with anti-μ in the spleen of a GF piglet.

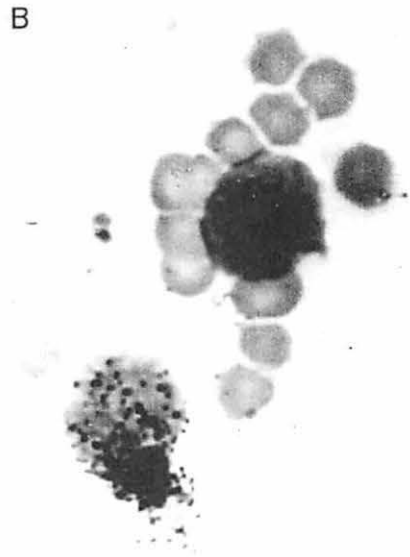

Fig. 2b A lymphocyte labelled with anti-γ from peripheral lymph nodes of GF piglets immunized with HSA-FA. Note that the grains are not localized over the cells. The E-rosetting cells are not labelled.

IgA were detected in concommitant experiments in all the organs tested (*Prokešová et al.*, 1976).

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