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Subpopulations of T lymphocytes emigrating in venous blood draining pig thymus labelled *in vivo* with fluorochome

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SUMMARY

The emigration of labelled thymus cells in the pig was studied directly in blood draining the large right distal cervical lobe of the thymus after controlled labelling with FITC delivered through cannulated branches of a main thymic artery and vein by temporary *ex vivo* perfusion at body temperature. Roughly 1% of thymic cells emigrated per day. Unlike most thymocytes, which are small, the size spectrum of thymic emigrants is slightly larger than that of typical blood lymphocytes. Surface-marker studies show that the surface phenotypes of the emigrants differ from both typical thymus and peripheral blood lymphocytes. Although the emigrants resemble thymocytes in the high proportion of strong rosettes formed with sheep red blood cells (RBC), they rosette poorly with pig red cells, particularly in the unenhanced saline test, in this respect behaving like blood lymphocytes. The peripheral T-cell subset bearing a Fc receptor is almost absent in thymus, but is well represented among the emigrants which thus resemble corticosteroid-resistant thymocytes in the pig. The large population of thymus-dependant Null lymphocytes in young pig blood apparently arise in thymus since they constitute 1/3 of emigrants, although only forming < 10% of thymus cells. This emigration of thymic cells is discussed in relation to its implications for the turnover of known functional peripheral T-cell populations.

INTRODUCTION

In many respects, the lymphocyte populations in pig blood are similar to those of man, but this economically important species shows notable differences in the classification and migration behaviour of its lymphocytes (Binns, 1982; Binns & Pabst, 1987). The subsets of sIg^+ B cells and E-rosette forming $CD2^+$ T cells (detailed below) are broadly conventional, but some 30–50% of blood lymphocytes in the young pig are Null T cells (Binns, 1982; Licence & Binns, 1984). However, the behaviour of these T-cell populations is paradoxical. On one hand, the Null T cells lack most T-cell properties (Binns, 1982) and yet disappear after thymectomy (Binns *et al.*, 1977), even though they form less than 10% of thymocytes (Binns, 1982). On the other hand, the E-rosette forming $CD2^+$ T cells, which show the classical T-cell involvement in immune responses (Outteridge, Binns & Licence, 1982; Binns, 1982), distribution (Binns, 1982; Denecke, Manuss & Trautwein, 1985) and recirculation behaviour (Binns, Pabst & Licence, 1986; Binns & Pabst, 1987), only show their thymus derivation following thymectomy combined with whole-body lympholytic treatments (Binns, 1980, 1982). This study aimed to investigate the thymus derivation of the Null cell, to confirm the thymus origin of E-rosetting T cells and to elucidate the maturational significance of the several subsets of E rosettes revealed by binding avidity for sheep and pig red blood cells (RBC) and for γFc-indicator cells.

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MATERIALS AND METHODS

The pigs in these studies, at Babraham, were three young Large Whites of 22–27 kg and one adolescent of 90 kg aged 3–6 months. The thymus in the pig lies both in the chest over the pericardium and along the jugular groove associated with the carotid artery, as far as the pharyngeal region. It is a large organ, weighing about 0·2% of body weight at 25 kg body weight (Pabst & Trepel, 1975). The region chosen to label *in situ* was the main mass of the right posterior cervical thymus, served by branches of a deep cervical artery, which in turn arises from the subclavian artery at the thoracic inlet. This region drains venous blood very variably by one or more small veins running into the internal jugular, external jugular and/or subclavian vein. In order to perfuse the organ, the thymic lobe and associated major vessels were approached under halothane general anaesthesia through a cervical ventral midline incision, using blunt dissection, and isolated from surrounding connective tissue, tying off minor blood vessels running to/from adjacent tissues. The small artery and vein providing the largest branch serving the thymic lobe was found, isolated, tied and cannulated towards the heart at a point cranial to the thymic vessels (Fig. 1a). This was

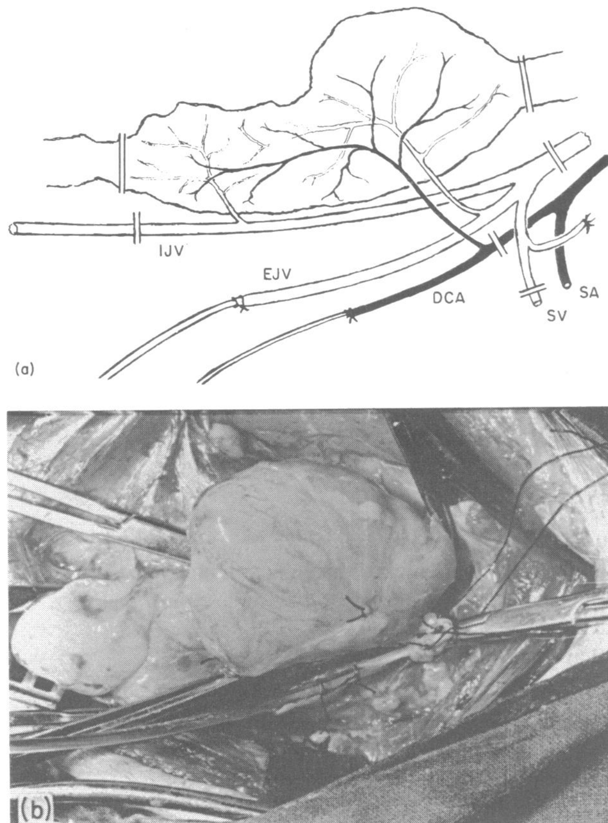

Figure 1. Diagram of the technique and anatomical landmarks in labelling of the right posterior cervical thymus by perfusion with FITC (a) The macroscopically normal lobe immediately after labelling (b) This diagram shows the thymic artery branching off the right deep cervical artery (DCA) and thymic veins from the right external (EJV) and internal jugular veins (IJV) cranial to the origins of the right subclavian artery (SA) and vein (SV). Parallel lines shows sites temporarily clamped off during the perfusion labelling via cannulas in the DCA and EJV.

normally the right deep cervical or thyroid artery and the external or internal jugular or subclavian vein. Vascular clamps were then placed on the main vessels centrally to the thymus branch, and the arterial cannula flushed with perfusion medium. The medium used was RPMI-1640 supplemented with 35 g/l each of two dexrans of MW 70,000 and 150,000. This was delivered from an autoclaved sterile perfusion system similar to that used to label pig spleen (Pabst & Binns, 1981) and lymph nodes (Binns, Pabst & Licence, 1985) with fluorochromes. This system comprises a silastic tubing circuit with a reservoir for 200 ml medium, a pump allowing adjustable flow, a membrane oxygenator, a bubble trap and a device for measuring perfusion pressure. As much of the circuit as possible was immersed in a water bath at 42°. The whole mobile assembly was brought close to the operation site and the arterial cannula connected. The residual blood was flushed out of the isolated thymus lobe and the effluent from the venous cannula discarded. The perfusion system was then stabilized to deliver 10–15 ml medium/min at 120 mmHg pressure and 39°. Sterile fresh autologous plasma and concentrated fluorescein isothiocyanate (FITC; Isomer 1 F5270, Sigma, St Louis, MO) in PBS were then added to the perfusion reservoir, to give concentrations of 1–2% plasma and 20–30 μg/ml FITC, and the circuit contents thoroughly mixed by rapidly circulating the medium via a temporary A-V shunt opened for this purpose. The thymus was then normo-thermically perfused with the labelling mixture for 15 min before washing rapidly with 20–50 ml fresh medium (Fig. 1b). The central arterial clamp was then removed and venous blood effluent flowing from its cannula collected continuously for 2–3 hr in timed heparinized aliquots. After this, the venous clamp was removed, the cannula filled with heparin-saline and shortened, the wound closed, leaving the cannula in the jugular groove, and the pig allowed to recover consciousness. The following day, the pig was re-anaesthetized, the central venous clamp replaced and thymic effluent blood collected from the venous cannula. The pig was then killed and samples of normal and labelled thymus taken for cell suspensions and histological study.

The collected thymic venous blood samples, pooled into a series of timed collections, were used to prepared lymphocyte suspensions, and the incidence of labelled lymphoid cells assessed as described previously (Binns, Pabst & Licence, 1981b; Pabst & Binns, 1981). The incidences of several subsets of T cells, of Fc-receptor bearing lymphocytes and of B cells among the labelled and unlabelled lymphocytes in the venous blood and in the thymus were studied using the following surface marker assays: E-rosette forming T cells formed with SRBC in dextran (DS, total E-rosetting population) and saline medium (S, T-cell subset rosetting strongly) (Binns, 1978), and with autologous pig red cells in 14% Ficoll (FP, T-cell subset rosetting with PRBC weakly) or saline (P, minor T-cell subset rosetting strongly with PRBC) using similar methods (Licence & Binns, 1984); Fc-rosette forming lymphocytes detected with pig antibody-coated agglutinable bovine RBC in saline (Fc, mainly B cells) or dextran (DFc, the extra number are mainly T cells) (Binns & Licence, 1981); total sIg$^+$ B lymphocytes detected by the direct antiglobulin rosetting reaction (DARR; Binns *et al.*, 1979a). The incidence of Null lymphocytes (which lack sIg and do not form E rosettes) was computed from the formula

$$\% \text{ Null} = 100\% - (\% \text{ DS} + \% \text{DARR}).$$

The samples used were from the last venous collection taken under anaesthesia (normally at 2nd–3rd hr) and a sample of normal thymus taken for the purpose.

The properties of the fluorescent lymphocytes in the 2–3-hr blood preparation and in the post-labelled thymus were analysed on a fluorescence-activated cell sorter (FACS 420, Becton-Dickinson, Mountain View, CA, settings 488 nM line, 400 mW laser power) assessing the fluorescence intensity and size profile (by forward scatter) of 10^4 cells in each population.

In view of the unexpected detection of Ig$^+$ cells among labelled thymus emigrants, the degree of uptake of plasma Ig onto autologous thymocytes was assessed. Samples of freshly prepared thymocytes were suspended in fresh autologous plasma for 30 min at 37° and then centrifuged on Ficoll–Triosil, washed and set up with indicator cells in the DARR B-cell assay, mimicking the processing steps that emigrating thymus lymphocytes are subjected to during their isolation from the thymic venous blood. As a control, an aliquot of control thymocytes was suspended instead in Eagle's minimum essential medium with 2·5% fetal calf serum (MEM 2·5% FCS, the usual suspending medium for marker assays) and kept on ice until included in the rosette assay.

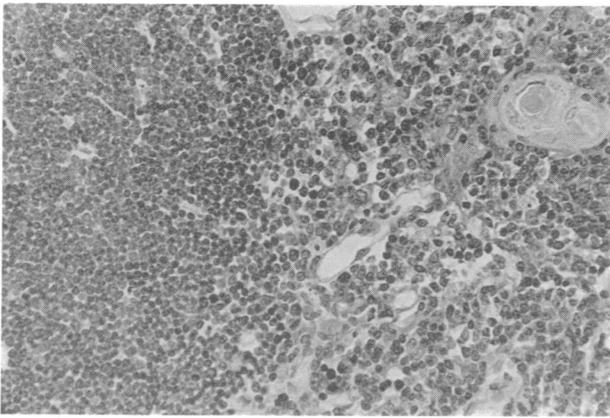

Figure 2. The essentially normal structure and preservation of cortex and medulla, Hassall's corpuscles and cortico-medullary blood vessels of the thymus 1 day after perfusion labelling with FITC. Giemsa, 2 μm stained section. Magnification × 240.

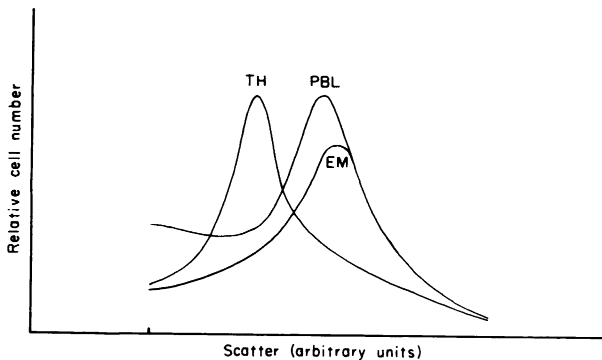

Figure 3. Diagram of the size profiles of labelled thymocytes (TH) and thymus emigrants (EM) and unlabelled blood lymphocytes (PBL) shown by forward scatter in the FACS 420, plotting scatter in 250 channels against relative numbers of cells in each channel.

Table 1. Proportion of lymphocytes in thymus, thymic emigrants and blood-bearing various subpopulation-specific surface markers

Subpopulation markers	Thymus	Thymic emigrants	Blood
%DS	90·6±1·7	61·2±7·3	41·5±7·9
%S	82·9±3·4	52·5±3·8	26·0±6·6
%DS-S	7·7±2·0	6·8±4·2	15·5±2·4
S/DS	0·92±0·02	0·88±0·06	0·61±0·05
%P	31·3±10·9	<0·14	0·15±0·01
%FP	56·6±9·5	19·6±3·2	14·2±4·4
P/DS	0·34±0·12	<0·010±0·001	0·004±0·001
FP/DS	0·62±0·10	0·32±0·03	0·32±0·09
%Fc	0·114±0·01	3·9±1·4	18·4±5·8
%DFc	0·21	13·2±3·7	37·1±6·4
%DFc-Fc	0·07	9·3±2·5	18·6±3·8
DFc-Fc/DS	0·001	0·18±0·07	0·50±0·11
%DARR	0·5±0·2	6·9±1·2	28·2±2·1
%Null	8·9±1·8	31·3±6·2	30·3±9·0

The techniques used in defining the proportion of cells in each subset (e.g. %DS, %FP, %DFc, %DARR, %NULL), the populations they define and their relative numbers (e.g. S/DS, DFc-Fc/DS) are described in the Materials and Methods section. The data are shown as mean ± SE.

RESULTS

Labelling procedure

Preliminary experiments demonstrated the feasibility of isolating and labelling the right posterior cervical lobe, which comprised about 20% of the thymus and showed that the first blood emerging from the thymus could contain up to 12% FITC-labelled cells. During the early operations, the level of label used for lymphocytes *in vitro* and for spleen perfusion *ex vivo* (~50 μg FITC/ml) proved unnecessarily high, tending to overlabel the afferent blood vessels and giving particularly strong labelling of thymocytes. For this reason 20–30 μg FITC/ml in a perfusion volume of 250 ml was used in most experiments and a protein labelling 'buffer' was included in the medium in the form of 1–2% fresh plasma or a little venous blood. Under these conditions the thymus segment weighing 10–20 g was labelled effectively and physiological blood flow maintained. Figure 1b shows the thymus labelled *in situ*. Although some oedema was found at the end of the label-washout procedure, this was predominantly in the interlobular tissue. Using this method, good tissue preservation was obtained (Fig. 2) and effectively all thymocytes were labelled at a level consistent with study for several days.

Export of labelled cells

The blood draining the labelled thymus lobe contained < 1–5% of labelled lymphocytes. The indices of labelled lymphocytes in the 2–hr sample that was used for assay of the properties of thymic emigrants in the four pigs were 3·0, 4·9, 2·3 and 1·3%, and even 20 hr later the incidence was still 0·8-1·6%. The labelling index in the peripheral circulation, estimated during the post-labelling collection, was neglible or very low, reaching 0·01% in one pig in which some thymic venous blood was allowed to escape to the central venous pool. However, overnight, when thymic blood was allowed to drain into the central pool, significant numbers of lymphocytes had migrated to peripheral lymphoid tissues. Thus, in one experiment the following labelling indices (%) were found at 22 hr in the peripheral blood and lymphoid tissues: blood, 0·06; spleen, 0·06; mesenteric lymph node (LN), 0·02; superficial inguinal LN, 0·02; retrosternal LN, 0·07; cervical LN, 0·04 (the latter two LN may drain some labelled thymus) and labelled thymus, 71·4. At 3 hr, the blood index was 0·007%.

Estimation of the rate of emigration of thymus cells in Pig 4, using the volumes and labelled-cell indices in the five samples of thymic venous blood, suggests that of the order of 1% of the number of cells in the thymus emigrate each day in a 26-kg pig, i.e. 10^9 cells/day or 2×10^7 cells/day/g thymus and 4×10^7 cells/day/kg body weight. This preliminary estimation is derived from the measured labelled lymphocyte indices (0·5–1·3%) and flow of blood, mean lymphocyte counts and the weight of perfused thymus and from published data (Pabst & Trepel,

1975) on relative thymus weight (0·22% body weight) and cell content. Pig 4 showed the lowest labelling index (0·5–1·3) and it is probable that the emigration in the other animals was similar or higher with indices in the 2–3-hr sample of 2·3–4·9%.

Size profile of labelled thymic emigrants

Study using the FACS of the volume and fluorescence profiles of the labelled thymic emigrant and thymus cells revealed several points. Essentially all (> 97%) of the thymus cells were labelled. Of these a large majority were very brightly labelled, in a way related to the FITC-concentration used. These studies confirmed the view from macroscopic assessments that 20–25 μg/ml FITC gave the best labelling level. As shown diagramatically in Fig. 3, the average volume of thymocytes was relatively small. When the level of fluorescence (shown by the channel with the largest number of cells or mode) was compared for the majority of small cells and the minority population of larger cells, the latter showed 2–3 times the average fluorescence intensity. In blood, two size profiles of unlabelled cells were present. The smaller cells were broadly similar to the majority of thymocytes, while the main peak was of larger lymphocytes. The great majority of labelled thymus-blood emigrants showed a size profile coincident with or in the larger half of the main blood lymphocyte peak. In general, the labelling level of the emigrants was lower than that of thymus lymphocytes. Thus it would appear that the major proportion of thymic cell emigrants are among the larger blood lymphocytes and of a size which constitutes a small minority of cells in the thymus, an organ which is composed predominantly of small lymphocytes.

Subpopulations among thymic emigrant cells

Study of the subpopulations of lymphocytes emigrating from the thymus compared to those in thymus and peripheral blood showed that each cell type has distinct features. Table 1 shows that among the E-rosetting T cells there is a distinct falling incidence of each subset during migration from thymus to the periphery. Thus the total E population (DS) forms 90% of thymocytes and these are predominantly strongly rosetting (S, 83%) with an S/DS ratio of 0·92. Many thymocytes also rosette with pig red cells in Ficoll (FP, 57%) and saline (P, 31%). Although the incidence of DS rosettes among thymus emigrants is lower than in thymus (61%), they too are strongly E-rosette forming with an S/DS ratio of 0·88. However, rosette formation with PRBC is much weaker among the emigrants, with indices of 20% in Ficoll and < 0·1% in saline. Regarding this second aspect, the E-rosette-forming emigrants are like normal blood lymphocytes which show indices of 14% and < 0·2%, since both lymphocyte types have FP/DS and P/DS ratios of 0·32 and < 0·01, compared to 0·62 and 0·34 in the thymus. However the emigrants differ from blood lymphocytes since the latter include a large proportion of weakly SRBC-rosetting cells (40%). Thus the S/DS ratio in blood is 0·61 (DS 41% and S 26%) compared with 0·9 for both thymus and thymus emigrants.

The three sources of cells also differ greatly in content of lymphocytes with a Fc receptor. In blood, past experience (Binns & Licence, 1981; Binns, 1982) has indicated that those found in saline (Fc) are mainly B cells while those requiring dextran enhancement of the test (DFcFc) are mainly E-rosette forming T cells. The present DFc and Fc results for blood are within the normal range, though the Fc value for Pig 1 is in the upper range. Typically thymus contains very few of either populations (0·14% Fc and 0·1% DFc-Fc) but the thymic emigrants include significant numbers of both, with 13% DFc and 4% Fc. Cells with immunoglobulin on their surface (DARR test) show a similar distribution difference with 28% in blood, 0·5% in thymus and 7% among thymus emigrants.

The thymus emigrants also appear more like blood lymphocytes regarding their Null cell content. While thymus contained only 9% Null cells, blood and thymus emigrants included 30% and 32%, respectively. Thus it seems that Null cells and the cells forming DFc rosettes in the thymus are among the more mature cells, being greatly enriched in the emigrating population, and that conversely, although strong SRBC-rosette formation does not reveal the state of maturity (S/DS = 0·9 in thymus and emigrants), rosette formation with PRBC may, since essentially none of the emigrants formed P rosettes while nearly 1/3 of thymocytes did so.

The small presence of sIg^+ (7·1%) and Fc^+ (3·7%) lymphocytes among the emigrants contrasted with the lack of these cells in thymus (0·5% and 0·1%). The possibility that the former might not be recirculating B cells but T cells with passively acquired intravascular Ig was shown by a mock blood cell preparation of thymic lymphocytes in the presence of fresh autologous plasma. These incubated thymic cells, which normally include < 1% sIg^+ cells, showed a $DARR^+$ incidence of 4% when incubated in plasma and centrifuged on Ficoll–Hypaque, suggesting that at least a proportion of the sIg^+ thymus emigrants may be T cells with passively acquired plasma Ig taken up by the Fc receptor or as antibody.

DISCUSSION

The aspects of these studies which warrant discussion are the techniques used, the data on the output and size profile of thymus emigrants and the significance of the phenotypic differences between thymocytes, thymus emigrants and peripheral lymphocytes for the understanding of the origin and maturation changes of pig lymphocyte subsets.

Several different methods have been used to study thymus cell emigration in various species, including thymus perfusion *in vitro*, arteriovenous differences in lymphocyte number, emigration of [^{3}H]thymidine-incorporating cells (Joel, Chanana & Cronkite, 1974) and tracing of fluorochrome-labelled peripheral lymphocytes after FITC injection into the thymus (Scollay, Butcher & Weissman, 1980). The present method combined aspects of several of these techniques, with our methods used previously to uniformly label lymphocytes with FITC in their normal micro-environment and estimate their direct emigration from pig spleen, lymph nodes, bone marrow, tonsil, and ileum (reviewed by Binns *et al.*, 1986; Binns & Pabst, 1987) and, in parallel with these studies, from sheep thymus (Yamashita, Miyasaka & Trnka, 1985). Although the optimum FITC concentration (20–30 μg/ml) was lower than that used for other organs (in spite of the use of a plasma protein buffer against over-labelling, also used for blood lymphocytes; Binns, Blakeley & Licence, 1981a), the straight-through perfusion labelling at physiological temperature, pressure and flow, easily monitored in this pale cream-coloured organ, was such as to give labelling levels that maintain cell viability and yet are sufficient to analyse the labelled-cell size profile on the FACS and to assess their

subset phenotypes within marker rosettes without altering the short-term distribution or phenotypes of FITC blood lymphocytes (Binns & Licence, 1985). Moreover, inclusion of both large and small molecular weight dextrans in the perfusate helped to reduce oedema and to maintain perfusion in this dense tissue, as we had found for lymph nodes (Pabst, Binns & Licence, 1985).

Estimation of the emigration rate from the thymus was not a prime purpose of these studies. However, the rough estimate of about 1% of thymocytes emigrating per day, calculated in the animal with the lower labelling indices in blood (0·5–1·3% versus 2·3–4·9% in the main sample from other pigs), is comparable with the 1% figure of Scollay *et al.* (1980) in the mouse and yet does not exclude the possibility that the emigration rates may have been higher, as reported in some other studies (reviewed by Joel *et al.*, 1974).

Our observations that thymic emigrants are larger than most thymocytes and on average tend to be among the larger peripheral blood lymphocytes, as assessed by forward scatter on the FACS, are also consistent with data in the mouse (Scollay, Wilson & Shortman, 1984).

As in other species, the phenotype of young pig thymus emigrants is intermediate between typical thymus and peripheral blood lymphocytes and resembles that of thymus medullary cells. Thus in their strong SRBC-rosette formation they resemble thymocytes, not peripheral T cells (which include up to 80% weakly rosetting cells; Binns, 1978). Yet, in their low level of PRBC-rosette formation (especially that without enhancement) and in the high level of γFc rosettes and of Null cells (four times the thymus level), they are like peripheral lymphocytes and not thymocytes, although similar to thymocytes remaining after the preferential stripping of thymic cortex by corticosteroid, which show increased Fc-rosette formation (Binns, 1982) and decreased PRBC-rosette formation (Salmon, 1983). These observed differences in the detailed phenotype of these cell types and the similarity of the emigrants with medullary and corticosteroid-resistant thymocytes is similar to that observed in rodents (Scollay *et al.*, 1984), while the maturational acquisition of Fc receptor and loss of PRBC binding is reminiscent, of the acquisition of the endothelial-binding receptor MEL14^{hi} during thymocyte emigration (Reichert *et al.*, 1984) and the loss of the B2A2 antigen within a few hours of emigration (Scollay *et al.*, 1984) in mice. Clearly the development and use of discriminating monoclonal reagents for pig T-cell subsets, not available during these studies, will greatly assist in the analysis of these maturation steps.

These studies are particularly informative about the origin of T-cell subsets. They show that the Null cells, which make up 20–50% and 4 million/ml of the blood lymphocytes in the young pig (Binns, 1982), are thymus derived, accounting for 1/3 of thymus emigrants but only 8% of thymocytes. This is in accordance with their disappearance after neonatal thymectomy (Binns *et al.*, 1977; Binns, 1982), their non-recovery after thymectomy and whole body irradiation (Binns, 1980) and their non-adherence for nylon-wool (Binns *et al.*, 1976b). It seems probable that these emigrating Null cells are not long-lived cells, since their numbers are such as to replace the blood Null cell pool in > 1 week. Initially this calculation appears logical, firstly because Null cells do not recirculate through lymph nodes or spleen (Binns, 1982; Binns *et al.*, 1985; Pabst *et al.*, 1985) and secondly because i.v. injected FITC$^+$ blood lymphocytes show increasing Null-cell proportions with time (Binns & Licence, 1985), suggesting that many of these cells stay in the circulation. However, recent data using ^{51}Cr-labelled blood lymphocytes suggest that large numbers of lymphocytes localize in lung, liver and bone marrow (Binns & Licence, 1985), at least in the former site, including Null cells which can re-emigrate (Binns *et al.*, 1986; Pabst *et al.*, 1987), and that at least some labelled Null cells localize in all the major sites of migration (Binns *et al.*, 1986). So realistic assessments of the average life span of Null cells are impossible.

These data also confirm the thymus origin of E-rosette forming lymphocytes, already indicated by their earliest appearance in fetal thymus and high incidence in postnatal thymus (Binns, 1982), their distribution in the lymph node paracortex and spleen white pulp (Denecke *et al.*, 1985), their slow recovery after thymectomy and lympholytic treatments (Binns, 1982), their T-like function in mitogen, antibody and cytotoxic responses *in vitro* (Outteridge *et al.*, 1982; Binns, 1982; R. M. Binns, unpublished data) and their carriage of a CD2-like 55,000 MW antigen revealed with polyclonal (Licence & Binns, 1985) and monoclonal antibodies (R. M. Binns, G. Butcher, D. Beale *et al.*, manuscript in preparation). Estimates of the replacement rate, and so life-span, of these cells are complicated too. Thus, if the thymus, which contains less than half the body's lymphocytes (Pabst & Trepel, 1975), exports 1% of its lymphocytes with an E-rosette content similar to the periphery (Binns, 1982), the emigrants should only replace the peripheral T-cell pool in »2 months and these cells must have a long average life-span. However, E-rosette forming T cells may also proliferate post-thymically since some cells incorporating [^{3}H]thymidine in the spleen are T cells (Pabst, Licence & Binns, 1983), and after neonatal thymectomy numbers of E-rosette forming cells in blood and most tissues (though not lymph nodes which show depressed lymphocyte traffic; Binns & Licence, 1985) are normal, in spite of the rapid growth of the pig (e.g. the blood lymphocyte pool increased >100-fold by 3–4 months). So it appears that E-rosetting T cells are replaced both from the thymus and from proliferation in other tissues and that, while they probably have a relatively long average half-life, this too may be a heterogenous property which should be investigated at the level of individual T-cell subsets, particularly in view of the functional immaturity of peripheral E-rosette forming T cells in thymectomized recipients (especially following recovery from whole body irradiation) both in maintenance of immunologcal integrity and recirculation behaviour (Binns, 1980, 1982; Binns & Licence, 1985).

The functional significance of the spectrum of subsets of T cells emigrating from the thymus is unclear. The possibility that both PRBC- and Fc-rosette formation are indicators of maturation state within the thymus was noted. However, the strength of SRBC binding clearly does not constitute a simple indicator of maturity. Strongly binding lymphocytes occur in thymus and among thymus emigrants (present data) but also predominate in the blood both of thymectomized (functionally deficient) and old pigs (mature) (Binns, 1982). Yet they also predominate among recirculating T cells emigrating from spleen, lymph nodes and lung (Binns *et al.*, 1981b, 1985, 1986; Pabst *et al.*, 1985), so this classification of T lymphocytes probably reflects their stable inactive state and is not an indicator of maturity since the weekly rosette-forming T cell predominates in the blood of young rapidly growing pigs (Binns, 1978) and is apparently less able to recirculate to lymphoid tissues.

On the other hand, strong rosette formation with autologous PBRC is rare both among thymus emigrants and in the blood, but accounts for 1/3 of thymocytes. So these cells appear to be relatively immature thymocytes, and this is in accordance with their relatively poor PHA and Con A responses when compared with thymocytes which rosette only with SRBC (Salmon, 1983).

The γFc-receptor bearing T cells, which are clearly greatly enriched among the emigrants are also enriched in corticosteroid-treated thymus (Binns, 1982; R. M. Binns and S. T. Licence, unpublished observations). In the periphery, the DFc rosettes are formed by Tγ cells (Licence & Binns, 1984), which may relate to putative Tγ-suppressor cells (Georgieva, 1984) and also to the Tc cells which bear the putative pig CD8 antigen (Pescovitz, Lunney & Sachs, 1985). So it is probable that the Fc^+ thymus cells emigrate as precursors of peripheral Tγ cells.

Initially the presence of sIg^+ lymphocytes among emigrant but not resident thymus cells might suggest the presence of B cells. However, since thymocytes processed in autologous serum acquire some sIg positivity, not noted for peripheral lymphocytes (Binns *et al.*, 1979a) and perhaps promoted by Ficoll–Triosil isolation (Alexander, Titus & Segel, 1978), it seems probable that most of the sIg^+ cells acquired their Ig during isolation from the thymic effluent blood: though the presence of a small number of B cells among lymphocytes recirculating through the thymus (Binns *et al.*, 1986) can not be ruled out.

Thus, these studies have demonstrated the feasibility of using controlled perfusion labelling of the thymus *in vivo* in larger animals to investigate the factors that influence the emigration of the young thymus-derived cell subsets which regenerate the peripheral T-cell pool and which are apparently responsible for so much of the inductive control, memory and effector function of the immune system. They promise to lay the foundation for future detailed study of the origin, maturation and functional properties of T-cell subsets in these economically important species.

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