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Immediate IgA precursor cells in rabbit intestinal lamina propria

A. D. BEFUS, M. O'NEILL & J. BIENENSTOCK *Host Resistance Programme, Department of Medicine, McMaster University, Hamilton, Ontario, Canada*

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Summary. Immunofluorescence studies have shown that injection of lymphocytes from either the intestinal lamina propria (LP) or from the Peyer's patches (PP) into irradiated (1000–1250 rad) allogeneic rabbits leads to the differentiation and proliferation of IgA containing cells in the spleen, mesenteric lymph node (MLN) and intestine by day 6 and to a lesser extent by day 4. In contrast, few IgA containing cells were seen in irradiated animals not given lymphocytes or given popliteal lymph node cells. Transfer of PP lymphocytes resulted in statistically greater numbers of IgA containing cells in the recipient MLN or spleen than did transfer of LP lymphocytes. In the PP of recipient rabbits given either LP or PP lymphocytes, intercellular IgA was abundant and more IgA containing cells were seen near the PP than in sites distant from PP.

These results show that IgA precursor cells are present in the intestinal LP as well as in the PP. The nature and distribution of the IgA precursor and the characteristics of the IgA repopulation are discussed.

INTRODUCTION

Since the observation that the predominance of IgA in mucosal secretions is in large part due to local production (Tomasi & Bienenstock, 1968), many investigators have attempted to understand the physiological basis of this association. Craig & Cebra (1971) made the important observation that 6 days after transfer of Peyer's patch (PP) cells into lethally irradiated, allogeneic rabbits the spleen and intestine were repopulated with IgA containing cells of donor origin. From this observation it was concluded that the PP represent an enriched source of immediate* IgA precursor cells. The appendix, another component of rabbit gut-associated lymphoid tissue (GALT), is a similar source of such precursor cells (Craig & Cebra, 1975) as is the bronchus-associated lymphoid tissue (BALT) (Rudzik, Clancy, Perey, Day & Bienenstock, 1975b). Moreover, repopulation with IgA containing cells occurs not only in the recipient spleen and intestinal lamina propria (Tomasi & Bienenstock, 1968; Rudzik, Perey & Bienenstock, 1975c), but also in the bronchial lamina propria (Rudzik *et al.*, 1975b). Whether enriched sources of immediate IgA precursors exist in other components of mucosal-associated lymphoid tissue is unknown.

Recently, a technique has been described for isolating lamina propria (LP) cells from rabbit small intestine and some of the characteristics of these

Correspondence: Dr A.D. Befus, Department of Medicine, McMaster University Medical Centre, 1200 Main Street West, Hamilton, Ontario, Canada, L8S 4J9.

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* This term is not in general usage but is used herein to distinguish precursor cells committed to IgA synthesis, as indicated by their surface IgA, from cells not committed to IgA such as surface immunoglobulin negative or surface IgM positive cells, although these may subsequently differentiate into IgA committed cells (see Discussion).

cells have been reported (Rudzik & Bienenstock, 1974; Singal, O'Neill, Clancy & Bienenstock, 1976). We report here upon the IgA repopulation characteristics and potential of PP and LP cells in the spleen, intestine and mesenteric lymph node (MLN) of irradiated allogeneic rabbits 4 and 6 days after transfer.

MATERIALS AND METHODS

Animals

Male New Zealand white rabbits, weighing on average 2 kg were given food pellets and water *ad libitum*. All animals to be irradiated received tetracycline hydrochloride (Tetralean, 1 g/l drinking water, M.T.C. Pharmaceuticals, Hamilton, Canada) from 24–48 h prior to irradiation until termination of the experiment.

Preparation of cells and their transfer

Pieces of rabbit small intestine freed of PP were used for preparation of LP cells. The rubbing technique previously described (Rudzik & Bienenstock, 1974) was used with little modification but for the use of Hanks's balanced salt solution (HBSS) with 10% foetal calf serum (FCS), pH 7·4, for the glass bead column and two subsequent washes of the cells. Gentamycin was not used in any of the media. The method yields approximately 150×10^6 viable (by trypan blue exclusion) LP lymphocytes from one rabbit small intestine, containing an average of 57×10^6 epithelial cells. These epithelial cells can be significantly depleted (at the expense of lymphocyte yields) by increasing the number of small glass beads in the column, by using slower elution rates from the column ($\leqslant$ ml/min), by centrifugation on Ficoll Hypaque or by passing the cell suspension over an additional cotton wool column in low ionic strength medium (VonBoehmer & Shortman, 1973).

Cell suspensions were prepared from excised, saline rinsed PP by disruption in a ground glass homogenizer with 5 ml of HBSS with 10% FCS, pH 7·4. Large and small debris were removed from the suspensions using the methods of Shortman, Williams & Adams (1972). The cells were washed twice at 4°, counted and the viabilities determined. This method yields $\geqslant 100\times10^6$ lymphoid cells per rabbit with viabilities in excess of 90%. Cells were prepared from popliteal lymph nodes (PLN) by similar methods.

Recipients of each cell type received 50×10^6 cells in 2 ml of medium via the marginal ear vein.

Irradiation

On the day prior to cell transfer, recipient rabbits were irradiated in pairs under pentobarbital anaesthesia. The animals were placed on a turn-table (6·8 rev/min) and the total body was exposed to a vertical beam of X-rays from a General Electric Maxitron 250 therapy unit at: 250 kvp, 30 mA, 0·5 mm copper, and 1·0 mm aluminium filter (half value layer 1·33 mm copper). The body dose was 850–1250 rad (see below) as measured at the mid plane, 90 cm from the centre line and at 42·5 rad/min.

Tissue processing

Four or six days after cell transfer (5 and 7 days post irradiation respectively), rabbits were killed by an overdose of pentobarbital and portions of spleen, MLN, ileum with the most distal PP and ileum without a PP were fixed and processed for tissue fluorescence as described previously (Rudzik *et al.*, 1975b, c). Single cell suspensions were prepared from the spleen and MLN using techniques described above for PP and PLN suspensions. Erythrocytes in spleen cell suspensions were lysed using ammonium chloride (Shortman *et al.*, 1972). Such suspensions prepared from irradiated recipients had lower viabilities (60–80%) than cells prepared identically from non-irradiated animals. Cytocentrifuge smears of the cells were prepared from 100 μl (4×10^6 cells/ml) of suspension at 500 rev/min in a Shandon cytocentrifuge for 10 min. The smears were fixed while still wet and subsequently stained and viewed within 48 h using the techniques and goat anti-rabbit heavy chain reagents described previously (Rudzik *et al.*, 1975b, c).

To determine the proportion of IgA containing cells, from 750–3400 (average 1650) cells were counted from each animal and the number positive determined. A form of Chi-square statistic, namely a 2×2 contingency table without Yates' correction factor was used to analyse the data (Remington & Schork, 1970) prior to standardization to IgA positive cells/10^3 total cells. This analysis was used to test if there was an association between treatment (e.g. donor cell source or recipient organ) and outcome (e.g. frequency of IgA positive cells) by

comparing the proportions of IgA containing cells in various recipients and their different tissues.

RESULTS

Normal rabbits

In normal rabbits, as is well established (Rudzik *et al.*, 1975b; Crandall, Cebra & Crandall, 1967), we observed numerous IgA containing cells in the LP of the intestine but saw few in sections of the spleen (< 1% IgA containing cells in smears). IgA containing cells represented 1–2% of the total in suspensions of MLN cells from normal animals.

Levels of irradiation

To determine the amount of irradiation which would be required to deplete the animals of IgA containing cells and to prevent endogenous repopulation with IgA containing cells during the experiments, rabbits were given 850, 1000 or 1250 rad but no cells and 7 days later were studied for IgA containing cells. The animals given 850 rad had 3–5% IgA containing cells in the MLN and spleen and numerous such cells in the LP of the bowel. In comparison with normal rabbits, rabbits given 850 rad had undergone some endogenous IgA repopulation. However, in rabbits given 1000 or 1250 rad IgA containing cells were rare in the LP and counts of cell suspensions of MLN and spleen showed 0–0·4% IgA containing cells (Table 1), with no difference apparent between 1000 and 1250 rad other than that 1250 rad was usually lethal within 10 days, whereas 1000 rad was seldom lethal even after 3 weeks. In all the experiments below, 1000 or 1250 rad was used.

IgA repopulation in cell recipients at 6 days

In contrast with animals irradiated but not given cells, by day 6 those animals given PP or LP cells had large numbers of IgA containing cells in smears of both spleen and MLN (Table 1). The morphology of the IgA containing cells in the smears varied from lymphocytes with a narrow rim of cytoplasm to typical plasma cells. Contamination of the donor population with from $2–50\times10^6$ epithelial cells

Table 1. Number of IgA containing cells per 10^3 cells in irradiated animals given 50×10^6 lymphocytes 6 days earlier

		Recipient organ	
Donor cell source	Animal	Spleen	MLN
X irradiated control	1*	0	4
	2	4	4
	3	1	4
Mean ± S.E.		1·7±1·2	4±0
Peyer's patches	1*	22	16
	2	44	115
	3	151	412
	4	13	101
Mean ± S.E.		57·5±31·8 [a, c] †	161±86·5 [a, d]
Lamina propria	1*	17	78
	2	62	47
	3	13	25
	4	28	70
	5	15	20
Mean ± S.E.		27± 9·1 [b]	48±11·6 [b, d]
Popliteal lymph node	1	10	6
	2	8	6
Mean		9	6

* Animals given 1000 rad of *X*-rays – others given 1250 rad.

† Values with the same superscript are significantly different ($P<0·0005$)

made no difference to the resultant IgA repopulation. The proportion of IgA containing cells in the MLN was significantly greater than in the spleen in recipients of both PP cells ($\chi^2=162{\cdot}5$, $P<0{\cdot}0005$) and LP cells ($\chi^2=49{\cdot}2$, $P<0{\cdot}0005$). Moreover, the proportion of IgA containing cells was greater in tissues of recipients of PP cells than LP cells; this difference was significant in the recipient MLN ($\chi^2=164$, $P<0{\cdot}0005$) and in the spleen ($\chi^2=31{\cdot}5$, $P<0{\cdot}0005$). IgA containing cells were rare in the spleen or MLN of irradiated controls or in rabbits given PLN cells (Table 1).

A study of IgG and IgM containing cells in the spleens or MLN of animals repopulated either with LP or PP cells was consistent with earlier reports (Craig & Cebra, 1971; Rudzik *et al.*, 1975b) on PP cells and showed that in the recipients, IgG and IgM cells were rare with either donor source.

On tissue sections of the spleen, clusters of IgA containing cells were seen surrounding the central vessels in the white pulp with some positive cells scattered in the red pulp, as has been shown previously (Craig & Cebra, 1971; Rudzik *et al.*, 1975b, c). In the MLN, IgA containing cells were found around follicles and in the medulla. The LP of rabbits which received LP or PP cells contained numerous IgA containing cells, whereas irradiated controls and rabbits given PLN cells had few IgA containing cells in the intestine. In IgA repopulated animals, positive cells appeared to be more abundant near PP. As reported previously (Rudzik *et al.*, 1975b), the PP and adjacent areas contained intercellular IgA. This was particularly clear when direct comparisons were made between tissues in or near PP and those selected from sites distant from a PP.

IgA repopulation in cell recipients at 4 days

As the intestinal LP is a major site of IgA plasma cells, it could be suggested that the IgA precursor in the LP is more mature than that in the PP and hence LP cells might repopulate recipients more rapidly than PP cells. To investigate this possibility irradiated recipients were studied 4 days after cell transfer (Table 2). IgA containing cells were seen in the spleen and MLN of both PP and LP recipients but it is clear that LP cells did not repopulate more rapidly than PP cells. Moreover, with both donor cell sources the IgA repopulation on day 4 was two to three fold less than that seen on day 6 (compare Tables 1 & 2).

Despite the quantitative difference between IgA repopulation on days 4 and 6, repopulation characteristics similar to those on day 6 occurred on day 4. For example, PP cells gave greater IgA repopulation than did LP cells in both the spleen ($\chi^2=13{\cdot}0$, $P<0{\cdot}0005$) and MLN ($\chi^2=150{\cdot}5$, $P<0{\cdot}0005$). In PP recipients the MLN was repopulated to a greater extent than the spleen ($\chi^2=40{\cdot}6$, $P<0{\cdot}0005$); this was not true of the LP however ($\chi^2=2{\cdot}7$, $P=0{\cdot}10$).

DISCUSSION

When allogeneic rabbits are irradiated and then given LP or PP cells, IgA containing cells proliferate

Table 2. Number of IgA containing cells per 10^3 cells in 1250-rad irradiated animals given 50×10^6 lymphocytes 4 days earlier

		Recipient organ	
Donor cell source	Animal	Spleen	MLN
Peyer's patches	1	4	11
	2	43	70
	3	34	65
Mean ± S.E.		27·0±11·8 [a, b] *	48·7±18·9 [a, c]
Lamina propria	1	25	27
	2	15	6
	3	1	26
	4	17	7
Mean ± S.E.		14·5±5·0 [b]	16·5±5·8 [c]

* Values with the same superscript are significantly different ($P<0{\cdot}0005$)

in the spleen, MLN and bowel within 6 days (Table 1). It is virtually certain that these IgA containing cells differentiate from donor cells, as irradiated control animals (1000–1250 rad) did not undergo endogenous IgA repopulation. Furthermore, the injection of allogeneic cells by itself is not a sufficient stimulus for IgA repopulation since allogeneic PLN cells did not lead to significant IgA repopulation. Moreover, studies with allotype antisera by Cebra and his colleagues have demonstrated that virtually all of the IgA containing cells which have differentiated and proliferated in the spleen and bowel of recipients are of donor orgin (Craig & Cebra, 1971; 1975). Hence, we have shown that LP cells represent an enriched source of immediate IgA precursors, as has been shown previously for PP, BALT and appendix (Craig & Cebra, 1975; Rudzik *et al.*, 1975b, c). It is clear that the PP are not the unique source of immediate IgA precursor cells.

Given the similarities between IgA repopulation with LP cells and with cells of the PP, BALT or appendix, it is likely that a similar precursor cell population exists in each lymphoid source. The precursor is not simply a plasma cell as such cells are rare in GALT and BALT (Waksman & Ozer, 1976) and in isolated LP cell suspensions (Rudzik & Bienenstock, 1974). The immediate IgA precursor in PP was identified in the surface μ negative light chain positive population (Jones, Craig, Cebra & Herzenberg, 1974) and subsequently was shown to be surface μ negative α positive (Jones & Cebra, 1974). α positive cells are abundant in PP (Jones & Cebra, 1974; Rudzik, Clancy, Perey, Bienenstock & Singal, 1975a) and also common in appendix, BALT and LP (Jones & Cebra, 1974; Rudzik *et al.*, 1975a), all of which have been studied using this IgA repopulation model. It would be of interest to investigate thoracic duct cells, as a relatively large proportion have surface α markers, (see below; Rudzik *et al.*, 1975a; Williams & Gowans, 1975).

The results of studies on day 4 after PP and LP transfer show that, with both donor cell populations, IgA repopulation occurs by day 4 as well as by day 6. LP gave less IgA repopulation than did PP cells on day 4 as on day 6, suggesting that the IgA precursor in the LP population is not more mature than that in the PP population. There appears to be a progressive accumulation of IgA containing cells in recipients rather than an abrupt appearance of such cells 6 days after transfer.

The precise location of the precursors in the LP remains to be determined; they may be scattered throughout the LP or concentrated in small isolated lymphoid follicles distributed throughout the bowel (Ham, 1969). A similar uncertainty applies to the BALT cells. Since in as yet unpublished studies we have shown in the guinea-pig that LP blast cells migrate differently from PP blast cells (the former selectively lodging in the bowel), it seems unlikely that IgA precursors in the LP are derived from small isolated follicles. Our observations that intercellular IgA was common in the PP follicles of animals repopulated with either LP or PP donor cells, and that more IgA cells were found near the recipient PP (as in normal animals, Crabbé, Nash, Bazin, Eyssen & Heremans, 1970), suggest that the precursors circulate through PP before arriving in the intestinal LP and completing differentiation.

PP cells produced a statistically significant greater number of IgA containing cells in the recipient spleen and MLN than did LP cells. In addition, the same statistical test, namely a 2×2 contingency table (Remington & Schork, 1970), used on the data of Rudzik *et al.*, (1975b), showed that PP cells produced significantly greater repopulation than did BALT cells. The simplest explanation for these differences is that the precursor cell (μ negative, α positive) is more abundant in the PP than in the LP or BALT. The data (Rudzik *et al.*, 1975a) support this explanation as surface α positive cells in the rabbit are most common in the PP, followed by the BALT, thoracic duct and then LP.

Whether the immediate IgA precursor is a virgin B cell, an antigen sensitized, or memory cell is uncertain. Sensitized cells committed to IgA antibody production are abundant in the MLN, thoracic duct and LP and may have been sensitized in the PP (Waksman & Ozer, 1976). The IgA proliferation in the spleen of irradiated allogeneic rabbits is due largely to an allogeneic effect (Rudzik *et al.*, 1975c). Therefore, it is likely that the immediate IgA precursor is not a virgin B cell since allogeneic effect factors are reported not to be effective in the induction of virgin cells but merely augment the proliferation of already sensitized cells (Feldman & Basten, 1972).

The fact that immediate IgA precursor cells are found in the LP demonstrates that all mucosal lymphocytes are not end-stage cells and that, therefore, lumenal antigen may directly influence their differentiation, proliferation and traffic.

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