Immunology 1977 33 727

Selective accumulation of cells with 'B' properties in stimulated lymph nodes

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Received 14 *February* 1977; *accepted for publication* 10 *March* 1977

Summary. Draining lymph nodes from mice which had been stimulated with bacterial adjuvants or the skin sensitizing agent, oxazolone, showed a marked increase in cell content, presumably due to lymphocyte immigration. A surprisingly large proportion of these cells exhibit properties of B lymphocytes: the presence of surface Ig, lack of Thy-1-like antigen and responsiveness to lipopolysaccharide (LPS). The relationship between the presence of surface markers and responses to class-specific mitogens, of cells from the stimulated nodes, was established by testing fractionated lymphocyte populations. Enriched T cells did not react to LPS, whereas removal of cells with Thy-1 antigen by specific antisera eliminated the reactions to T mitogens but had little or no effect on the LPS response. The data thus suggest that B cells, which make up a small portion of the circulating lymphocyte pool, are selectively accumulated in lymph nodes stimulated by different immunogens, including T-specific stimulants. This interpretation contradicts the generally accepted assumption, that stimulated lymph nodes trap mostly T lymphocytes.

INTRODUCTION

Local administration of antigens brings about a rapid increase in size and cellular content of draining lymph nodes. Studies of Hall (1974), Zatz & Lance (1971), Cahill, Frost & Trnka (1976) and others have shown that the increase in cell content results mostly from the accumulation of circulating lymphocytes in the stimulated node. The use of adjuvants was found to prolong and intensify the magnitude of this cellular reaction (Taub & Gershon, 1972; Lance & Frost, 1974). Based mainly on indirect evidence (e.g., Taub & Gershon, 1972), it has been generally assumed that most of the accumulating cells are circulating T lymphocytes. We show here, however, that a large proportion of the cells found in stimulated lymph nodes exhibit properties of B cells. The agents used for the lymph node stimulation included various bacterial adjuvants, as well as the skin sensitizing hapten, oxazolone, which stimulates mainly T lymphocytes (Davies, Carter, Leuchars & Wallis, 1969; Davies, 1969; Kerbel, Elliott & Wallis, 1974).

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MATERIALS AND METHODS

Sensitization of mice

Male or female C57Bl/6 mice, 8–14 weeks of age, were obtained from the animal breeding farm of this School, or from Jackson Laboratory, Bar Harbor, ME. Groups of mice were stimulated with either one of the following agents: (a) approximately

5×10^8 killed, thrice washed *Bordetella pertussis* organisms (kindly supplied by Rafa Laboratories, Jerusalem); (b) 0·05 ml of a suspension of *Corynebacterium parvum* CN6134, equivalent to 0·35 mg dry weight organisms (kindly provided by Wellcome Laboratories, Beckenham, England, batch PX 374); (c) 0·5 mg of methanol extraction residue (MER) of Bacillus Calmette Guerin organisms (Gery, Baer, Stupp & Weiss, 1974); or (d) 0·2 ml of a 3% ethanol solution of the skin sensitizing chemical oxazolone (4-ethoxymethylene-2-phenyl oxazolone) (kindly given by Dr A. J. S. Davies of the Chester Beatty Research Institute, London). The adjuvants were injected into both fore footpads, in a total volume of 0·05 ml, while the oxazolone solution was painted over the shaved chest and fore footpads.

Preparation of cell suspensions

At various intervals after treatment, the axillary lymph nodes were removed, pooled within the group and teased gently apart. Axillary or mesenteric nodes from untreated mice were used for controls, as detailed below.

Determination of cells with T or B markers

Thy-1 antigen or surface immunoglobulin (sIg) were used as markers for T or B cells, respectively. The presence of Thy-1-like antigen was determined by the cytotoxic effect (Mugraby, Gery & Sulitzeanu, 1974a) of properly absorbed rabbit antiserum against mouse brain-associated Thy-1 antigen ('anti BAT') (Golub, 1971), while sIg was detected by the indirect immunofluorescence method using rabbit antiserum against mouse Ig and fluorescent goat antiserum against rabbit IgG (Mugraby, Gery & Sulitzeanu, 1974a).

Enrichment of T and B lymphocytes

T cells were purified by passing the lymph node cell suspensions through degalan columns coated with mouse Ig and rabbit antibodies to mouse Ig ('Ig columns') (Mugraby *et al.*, 1974b). B lymphocytes were enriched by treating the suspensions with either the anti-BAT serum or allogeneic antiserum to Thy-1·2 (AKR anti C3H, kindly given by Dr R. S. Kerbel, of Queen's University, Kingston, Ontario), followed by guinea pig complement (Mugraby *et al.*, 1974b).

Mitotic responses in culture

The cultures were set up, in duplicate, in 17 × 100 mm plastic tubes (Falcon, Oxnard, CA) and consisted of 2×10^6 unfractionated lymph node cells, or 10^6 enriched populations, in 1 ml RPMI-1640 medium, with 5% heat-inactivated normal human serum. The mitogens were added in the following doses: phytohemagglutinin P (PHA, Difco, Detroit, MI), 0·5 μl; concanavalin A (Con A, Miles-Yeda, Rehovot), 2 μg; lipopolysaccharide W from *E. coli* 055 : B5 (LPS, Difco), 50 μg. The cultures were incubated and their responses determined, by the uptake of [^{3}H]-thymidine, as described elsewhere (Ron, Laufer & Gery, 1973). The results are presented as mean c.p.m. of the duplicate cultures, which differed from the individual values by less than 15 per cent.

RESULTS

Characterization of cells in stimulated lymph nodes

Axillary lymph nodes collected 4 days after treatment with the adjuvants, or 3 days after painting with oxazolone, contained 2–5 times more cells than found in their untreated controls. The proportions of cells with the T- or B-markers in lymph nodes from the different mouse groups are shown in Fig. 1, which summarizes repeated experiments. All treated

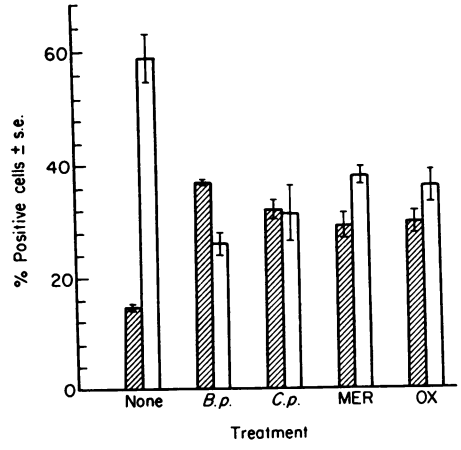

Figure 1. Effects of treatment with different agents on the proportion of 'B' or 'T' lymphocytes in the draining (axillary) lymph nodes. The agents were: *B. pertussis* (*B.p.*) and *C. parvum* (*C.p.*) organisms, the 'MER' fraction of BCG and oxazolone (OX). Each bar represents the mean value ± S.E. of at least three repeated experiments. ▩ Ig coated ('B') cells; □ Thy-1 ('T') cells.

Table 1. Mitotic response of lymph node cells from mice treated with different agents*

Treatment of mice	Mitogen in culture			
	None	PHA	Con A	LPS
Untreated	957 ± 103†	50,856 ± 4616	113,285 ± 15,093	5483 ± 159
B. pertussis	11,386 ± 299	68,016 ± 17,664	122,670 ± 20,211	46,608 ± 5544
C. parvum	7038 ± 1941	50,194 ± 20,531	123,647 ± 25,901	21,063 ± 995
MER	3139 ± 1303	62,261 ± 252	118,641 ± 23,116	13,321 ± 1479
Oxazolone	12,247 ± 1459	47,220 ± 8868	85,233 ± 7835	33,323 ± 3877

* Axillary nodes from the stimulated nodes and a combination of axillary and mesenteric nodes from the untreated controls were used in these experiments.

† Incorporation of [^{3}H]-thymidine, presented as mean cpm of two separate experiments ± s.e.

animals exhibited a reduction in the percentage of cells with Thy-1-like antigen and a corresponding increase in the proportion of Ig-coated cells. A similar pattern of changes was found in mice examined 2 days after treatment with the various agents (not shown here).

Mitotic responses of cells from stimulated nodes

Cells from stimulated nodes differed also from their controls in their mitotic responses *in vitro* (Table 1). Cultures from all treated groups incorporated strikingly more thymidine than those from untreated controls when incubated without mitogen ('spontaneous uptake') or with the B-specific mitogen, LPS (Gery, Krüger & Spiesel, 1972). No significant difference was found, however, between the response of cultures from treated and control animals to the T-specific mitogens, Con A or PHA (Stobo, Rosenthal & Paul, 1972).

Mitotic responses of enriched lymphocyte populations

The relationship between the responsiveness towards class-specific mitogens and the presence of cell markers was examined with enriched populations of T or B cells from oxazolone-treated or control mice. Two typical experiments are shown in Table 2. T cells from either normal or treated donors reacted like the unfractionated suspensions against PHA or Con A, but did not respond to LPS and exhibited very low 'spontaneous' incorporation. Killing of the cells that carry Thy-1-antigens, on the other hand, eliminated the reactions to the T-specific mitogens

Table 2. Mitotic responses of enriched T or B cells from untreated or oxazolone-stimulated mice

Exp. no.	Stimulation of mice	Treatment of cells	Mitogen in culture			
			None	LPS	PHA	Con A
I	None	None	502	1548	48,647	108,603
		Ig column	314	645	62,958	103,367
		Anti BAT	1058	5455	1545	1417
	Oxazolone	None	11,027	30,009	91,737	117,260
		Ig column	179	317	68,402	90,147
		Anti BAT	5367	31,340	3706	6364
II	None	NMS*	299	5469	34,386	92,555
		Anti Thy-1·2	334	2297	233	1255
	Oxazolone	NMS	9343	43,648	37,318	109,195
		Anti Thy-1·2	1518	27,123	726	8629

* Normal mouse serum.

of both groups of mice, but had little effect on the response to LPS. Treatment with the antibodies to BAT or Thy-1·2 reduced considerably, however, the high 'spontaneous' incorporation of cells from the oxazolone-stimulated lymph nodes.

DISCUSSION

The increase in cellular content of the stimulated lymph nodes, noted here, is assumed to result mostly from the accumulation of circulating cells. The emigration of lymphocytes into similarly activated lymph nodes was established by other authors (see Introduction), while local cell proliferation could not contribute significantly to this process, since it starts about 3 days following stimulation with various antigens (Davies, 1969).

Our data show a surprisingly large proportion of cells with B-properties in lymph nodes stimulated with the bacterial adjuvants, or even with the skin sensitizing, T cell-activating agent, oxazolone. The identity of the accumulating cells was based on both their cell surface markers and responsiveness toward class-specific mitogens. Thus, the stimulated nodes had more cells with sIg, but less with Thy-1 antigen than their unstimulated controls. In other experiments, carried out with Dr R. S. Kerbel (Queen's University, Kingston, Ontario), an increase was also found in the proportion of cells with Fc receptors in oxazolone-stimulated lymph nodes (to be reported elsewhere). In culture, the cells from the stimulated nodes reacted well to the B-specific mitogen, LPS, while normal lymph node cells react poorly to this mitogen (Peavy, Shands, Adler & Smith, 1973 and this report).

The accumulation of cells in the stimulated lymph nodes reported here is analogous to the 'trapping' phenomenon, described by Lance, Taub and their colleagues (Zatz & Lance, 1971; Taub & Gershon, 1972; Lance & Frost, 1974). Our finding of a selective accumulation of cells with B properties contradicts, however, the assumption of the mentioned investigators, that T cells are exclusively those 'trapped' by the activated nodes. This assumption has been based mainly on indirect evidence: the finding of accumulating cells in the T-dependent paracortical area of the nodes and the lack of such response in thymus-deprived mice (Taub & Gershon, 1972). Histological examination of the stimulated nodes showed in our study, too, an intense hyperplasia of the paracortical areas (unpublished observation). The possible discrepancy between these histological findings and the increase in cells with B properties in the stimulated nodes could be reconciled in view of the finding of Lance & Frost (1974), that the paracortex serves as the major trapping site. Thus, B cells, too, may be trapped in this 'T-dependent' area. If this were the case, one might attribute the lack of migration of cells into lymph nodes of T cell-deprived mice (Taub & Gershon, 1972) to the loss of trapping capability in such animals (Zatz & Gershon, 1974).

An alternative interpretation to our results could be, on the other hand, that many of the cells with B properties in the stimulated nodes were originally T-cells and acquired these properties due to their vigorous activation *in vivo*. A number of recent reports have shown that activated T cells exhibit B markers such as sIg or Fc receptors (Pernis, Miller, Forni & Sprent, 1974; Krammer, Hudson & Sprent, 1975; Stout & Herzenberg, 1975). In most of these studies, however, T markers were found on the activated cells, along with the B markers, while in our study, no evidence was found to support the existence of such cells in great numbers: the increase in sIg^+ cells was accompanied by a decrease in $Thy\text{-}1^+$ lymphocytes (Fig. 1) and antisera to Thy-1 had little or no effect on the response to LPS (Table 2).

Both anti BAT and anti Thy-1·2 sera reduced considerably the level of 'spontaneous' thymidine incorporation in cultures from the oxazolone-stimulated nodes. There are reported data indicating that the spontaneous response in normal lymphocyte cultures is produced mainly by B cells (Wybran, Chantler & Fudenberg, 1973; Hedfors, 1974). In the oxazolone-stimulated nodes, however, T cells were expected to play a major role in bringing about the increased spontaneous thymidine incorporation, as a consequence of their selective activation *in vivo*. Indeed, most proliferating cells in these nodes were shown to be T cells (Davies, 1969) and to carry Thy-1·2 antigen (Kerbel *et al.*, 1974).

Our finding of selective accumulation of cells with B properties in activated lymph nodes is in accord with recent reports showing B cell emigration to nodes stimulated by haemocyanin (Brahim & Osmond, 1976; Green & Fanger, 1976) or allogeneic lymphoid cells (Brahim & Osmond, 1976). It seems, therefore, that the selective trapping of B cells is a common feature of the lymph node reaction to

different antigens and may be a major component of the recruitment process (Hall, 1974). It is noteworthy that oxazolone, which activates mainly T cells (see above) provokes the selective accumulation of lymphocytes with B properties; this may indicate that the trapping process is unrelated, at least in part, to the cellular immune response which follows the exposure to antigen.

The finding that more B cells than T cells are accumulated in the stimulated lymph nodes may indicate the existence of a highly selective mechanism of trapping. This level of selectivity seems essential in view of the fact that B cells make up a mere third or less of the circulating lymphocyte pool (Raff, 1971).

ACKNOWLEDGMENTS

We thank Drs R. S. Kerbel and D. W. Weiss for helpful discussions. This study was supported by grant no. 82 from the U.S.–Israel Binational Science Foundation.

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