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CHANGES IN LYMPHOCYTE ADHESIVENESS DURING CONTACT SENSITIZATION

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Summary.—Lymphoid cells from the draining lymph nodes of mice 3 days after oxazolone application were found to be more adhesive to each other and to glass than cells from unsensitized nodes.

Examination of the temporal sequence of changes during contact sensitization showed that during the first 4 days after oxazolone application there was an increase in both the cellularity of the draining node and in the intercellular adhesiveness of cells from the draining node, which persisted for at least 14 days. There was also an increase in the lymphoblast content; however, this was transient, peaking at Day 4 before returning almost to normal by Day 7.

The intercellular adhesiveness of a lymphoblast-enriched subpopulation from the draining node was greater than the adhesiveness of a small lymphocyte subpopulation from the same node. However, the adhesiveness of both these subpopulations were greater than that of normal, unsensitized lymphoid cells.

The implications of a change in cellular adhesiveness on the migratory properties *in vivo* of lymphocytes are discussed.

CONTACT SENSITIZATION with simple chemical compounds results in several cellular and morphological changes both in the skin exposed to the sensitizing agent and in the lymph node draining the exposed skin. Thus, mononuclear cell infiltrates have been observed in the skin of guinea-pigs exposed to dinitrochlorobenzene (DNCB) and skin thickening has been seen to occur 18–48 h after sensitization (Flax and Caulfield, 1963; Turk, Heather and Diengdoh, 1966). Furthermore, large pyroninophilic lymphoblasts appear in the paracortical region of the draining lymph nodes reaching a peak 3–4 days after sensitization, but subsiding by 7 days after sensitization.

Similar changes have been found in the skin and draining lymph nodes of mice in response to topically applied oxazolone (Asherson and Ptak, 1968). Parrot and de Sousa (1966) demonstrated that the appearance of the pyroninophilic lymphoblasts in response to oxazolone was thymus-dependent and they suggested that contact sensitivity depends on the recirculating lymphocyte population. Davies *et al.* (1969) showed that the response to oxazolone was biphasic. They noticed that the initial proliferative response peaking around 3–4 days was thymus-dependent while the subsequent proliferative response peaking around Day 8 was thymus-independent.

During the response to oxazolone the cellularity of the draining lymph node increases to about 4 times the normal level (Davies *et al.*, 1969). Asherson and coworkers (Asherson and Allwood, 1972; Asherson and Barnes, 1973), investigating contact sensitivity to picryl chloride and oxazolone in mice, demonstrated that the

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net arrival of lymphocytes to the draining lymph node was 4–8 times greater than that to the non-draining node, with a peak in the number of cells localizing to the draining node 4 days after sensitization. Thus the increase in cellularity of the draining nodes during contact sensitization would seem to be due to a combination of an increase in the number of recirculating cells migrating to the node and an increase in the proliferation of cells in the node in such a way that arrival and production exceed release of cells from the node.

Here we report a change in the properties of lymphoid cells during contact sensitization which may contribute to changes in cellularity, namely an increase in the adhesiveness of lymph-node cells in response to oxazolone.

MATERIALS AND METHODS

Oxazolone sensitization.—Oxazolone (4 ethoxymethylene-2-phenyl oxazolone, B.D.H.) was dissolved at 30 mg/ml in acetone. One drop (approximately 30 μl) was applied topically by means of a Pasteur pipette either on to the ear or on to the shaven flanks of C57Bl mice and allowed to dry.

Preparation of lymphoid cells.—Animals were killed by prolonged ether intoxication. Lymph-node cell (LNC) suspensions were prepared after excision of the inguinal or cervical lymph nodes. After trimming off the excess fat these nodes were teased apart in ice-cold RPMI 1640 medium (Gibco Biocult) buffered to pH 7·4 with 10^{-2}M HEPES (N-2-hydroxyethylene-N^1-2-ethane sulphonic acid). The resulting cell suspension was washed 3 times by centrifugation and resuspended at 10^7 cell/ml in HEPES-buffered RPMI 1640. Before use the cell suspension was filtered through 20μm nylon mesh (Begg Cousland Ltd.) to remove debris and cell clumps. Cell counts were performed using a Coulter electronic particle counter (model ZB) linked to a Channelyzer (model C1000) or by using an improved Neubauer haemocytometer.

Cell aggregation.—The aggregation of lymphoid cells was performed in co-axial Couette viscometers constructed by Barholm Tool and Gauge Co. Ltd., Glasgow. When rotated these viscometers maintain a laminar shear flow under known conditions. The lymph-node cell suspensions were adjusted to a fractional volume of $6{\cdot}86 \times 10^{-2}$% ($5 \times 10^6$ cells/ml of normal lymphocytes) and 0·8 ml of this suspension was introduced into the gap between the viscometers, which were then rotated at a shear rate of 10 sec^{-1}. The collision efficiency (the probability that a collision between 2 cells will result in adhesion) was then calculated as originally described by Curtis (1969) from:

$$\ln \frac{N\infty t}{N\infty o} = -\frac{4\Phi\alpha tG}{\pi}$$

where N∞o is the number of particles at the start of aggregation, N∞t the total number of particles (single cells plus aggregates) at time t, Φ is the fractional volume of cells and G is the shear rate. The viability at the end of the experiment, as determined by trypan-blue exclusion, was consistently >80%.

The measurement of cell-substrate adhesion.—The adhesion of lymphoid cells to a glass substrate was measured using chambers constructed from circular 13mm diameter coverslips and a Millipore diffusion chamber ring as described by Kellie, Evans and Kemp (1980).

Lymph-node cellularity.—Lymph-node cellularity was assessed by teasing the node apart in a standard volume of medium and counting in a Coulter counter.

Differential cell counts.—Differential cell counts were performed on Jenner–Giemsa stained preparations from a Cytospin. At least 200 cells in each fraction were examined.

Density-gradient separation of cells.—Percoll (r) was diluted 9:1 with ten-fold concentrated Dulbecco's phosphate-buffered saline (PBS). This solution was then diluted with single-strength PBS to the required concentrations. A discontinuous density gradient was made by carefully layering 4 ml of 62%, 55%, 48%, 41% and 34% Percoll (r) solutions on top of one another into a 30 ml centrifuge tube. 5×10^7 cells in 4 ml of 19% Percoll (r) were then applied to the top of the gradient and the tube was centrifuged at 800 *g* for 30 min at 25°. The cells at the density interfaces (Fractions I–V) and the pellet (Fraction VI) were collected using a syringe with a wide-bore needle, washed 3 times in medium, and separate samples were counted, stained or used to measure intercellular adhesiveness.

RESULTS

The adhesiveness of cells after oxazolone sensitization

The cell–cell adhesiveness of lymph-node cells 3 days after the application of oxazolone was measured. The collison efficiency of normal lymph-node cells was $12{\cdot}39 \pm 0{\cdot}62$%, whereas the collision efficiency of lymph-node cells 3 days after sensitization with oxazolone was $22{\cdot}31 \pm 1{\cdot}58$%. There was no significant difference

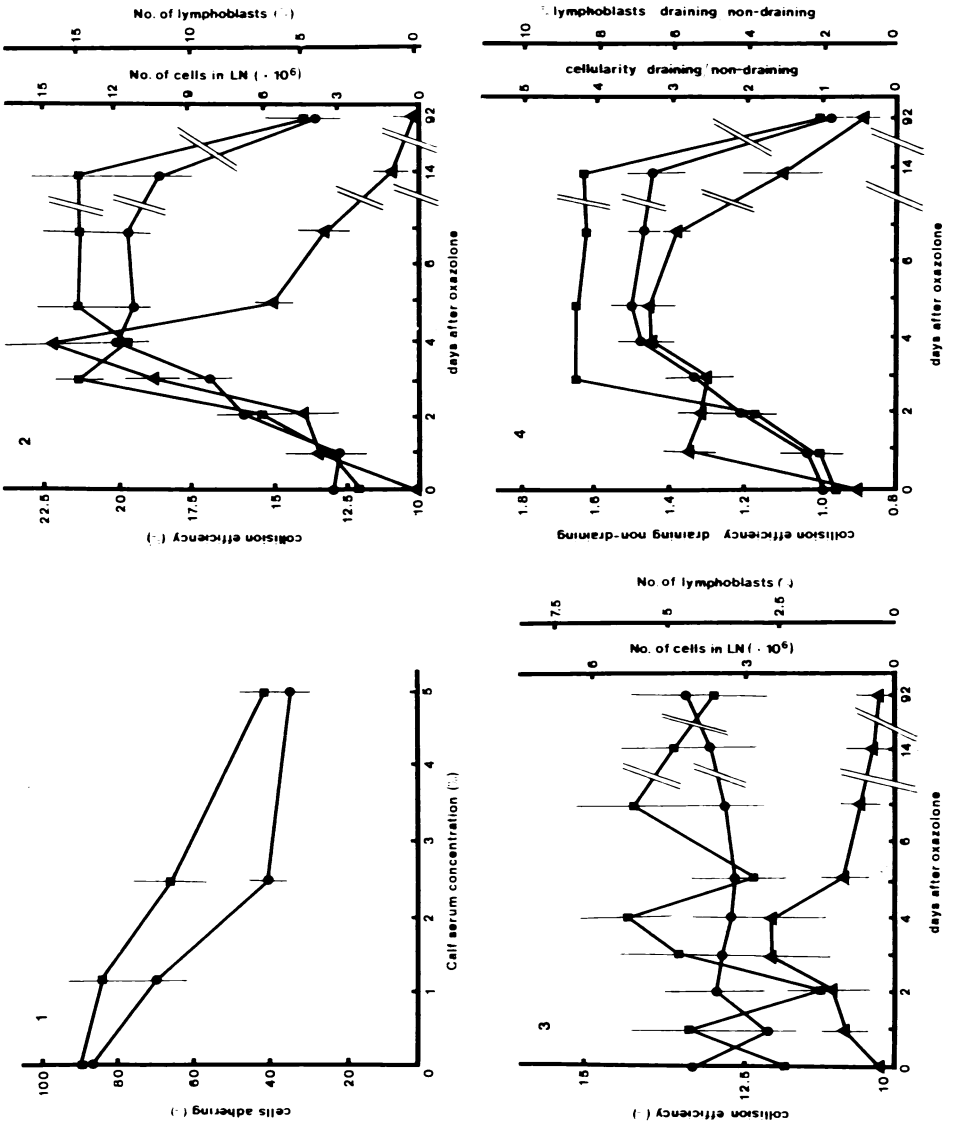
No. of lymphoblasts draining non-draining
cellularity draining/non-draining
collision efficiency draining non-draining
No. of lymphoblasts ( )
No. of cells in LN (· 10⁶)
collision efficiency ( )
days after oxazolone
No. of lymphoblasts ( )
No. of cells in LN (· 10⁶)
collision efficiency ( )
days after oxazolone
Cell serum concentration ( )
cells adhering ( )
1
2
3
4

TABLE I.—*The cell–cell adhesiveness of normal and oxazolone-sensitized lymph node cells 4 days after sensitization*

Cells	Collision efficiency* % ± s.e. mean	n	Significance
Normal LNC	12·39 ± 0·62	15	
Normal LNC + 50 $\mu g/ml^{-1}$ oxazolone	13·91 ± 1·44	10	$P > 0·1$
3 day oxazolone-stimulated LNC	22·31 ± 1·58	15	$P < 0·05$

* Curtis (1969).

in the adhesiveness of cells aggregated in the presence of 50 $\mu g/ml$ oxazolone from cells aggregated in its absence (Table I). Thus the increase in the adhesion of lymphoid cells sensitized *in vivo* with oxazolone is likely to be due to activation of these cells rather than to interaction of oxazolone with the cells *per se*.

In the presence of calf serum, which inhibits the substrate adhesion of lymphoid cells (Kellie *et al.*, 1980), the adhesion of oxazolone-sensitized cells to glass was greater than the adhesion of normal cells (Fig. 1). Thus the intercellular and substrate adhesion of lymph-node cells is raised during contact sensitization.

The temporal sequence of events during contact sensitization

To investigate more fully the relationship between adhesiveness and sensitization, mice were painted on the right ear with oxazolone and at various times the draining cervical and contralateral (non-draining) lymph nodes were excised and their cellularity, lymphoblast content and intercellular adhesiveness were measured.

The cellularity of lymph nodes after oxazolone sensitization.—The number of cells in the draining lymph nodes rose from $3·49 \pm 0·34 \times 10^6$ (s.e. mean, n = 3) to $12·08 \pm 1·16 \times 10^6$ during the first 4 days and remained elevated for at least 14 days after painting, returning to normal by 92 days after oxazolone application (Fig. 2). There was no significant difference in the cellularity of the contralateral lymph nodes at any of the times tested from the cellularity at Day 0 (Fig. 3, $P > 0·05$). Cytospin preparations showed that the cells were > 90% lymphoid cells.

The blast cell response of lymph nodes after oxazolone sensitization.—The proportion of lymphoblasts in the draining lymph nodes increased from 0·16 ± 0·19% (s.e. mean, n = 3) at Day 0 to 14·8 ± 0·9% at Day 4 before declining almost to normal at Day 14. By Day 92 there was no significant difference in the proportion of lymphoblasts from the Day 0 value (Fig. 2). In the contralateral nodes there was a slight increase in the number of lymphoblasts from 0·14 ± 0·12% to 2·64 ± 0·62% which then declined (Fig. 3).

The adhesiveness of lymph-node cells after oxazolone sensitization.—The adhesiveness of cells from the draining lymph nodes increased during the first 3 days after oxazolone application from 12·22 ± 0·88% (s.e. mean, n = 20) to 21·32 ± 1·58%. The collision efficiency remained at this level for at least 14 days after sensitization (Fig. 2). By 92 days after sensitization the collision

FIG. 1.—The substrate adhesion of lymphoid cells 3 days after oxazolone sensitization. The number of cells adhering to a glass coverslip in the presence of calf serum was counted using phase-contrast microscopy. ●—●, Normal lymph-node cells; ■—■, lymph-node cells 3 days after oxazolone sensitization.

FIG. 2.—Temporal sequence of changes of adhesiveness, cellularity and blast-cell number in draining lymph nodes during contact sensitization caused by oxazolone. ■—■, Adhesiveness (collision efficiency, %); ●—●, number of cells in lymph node (× 10^6); ▲—▲, proportion of lymphoblasts in lymph node (%).

FIG. 3.—Temporal sequence of changes in adhesiveness, cellularity and blast-cell number in non-draining contralateral lymph nodes during contact sensitization caused by oxazolone. ■—■, Adhesiveness (collision efficiency, %); ●—●, number of cells in lymph node (× 10^6); ▲—▲, proportion of lymphoblasts in lymph node (%).

FIG. 4.—Adhesiveness, cellularity and blast-cell ratios of draining *vs* non-draining lymph nodes during contact sensitization caused by oxazolone. ■—■, Adhesiveness: collision efficiency draining/non-draining; ●—●, cellularity: draining/non-draining; ▲—▲, % lymphoblasts: draining/non-draining.

TABLE II.—*Differential cell counts of fractions obtained by density centrifugation in a discontinuous Percoll (r) gradient*

Fraction	Large blasts %	Medium lymphocytes %	Small lymphocytes %	Monocyte/ macrophages %	PMNs %	Unidentified or damaged %
I	4·0	5·4	42·9	23·8	22·0	2·9
II	19·8	7·8	40·0	20·8	7·2	4·4
III	37·5	11·1	22·9	14·0	7·8	6·3
IV	43·4	5·5	33·2	8·8	6·3	3·8
V	4·1	8·8	67·1	8·0	7·4	4·6
VI	4·6	2·9	77·3	7·9	4·4	2·9

efficiency had decreased down to unstimulated levels (Fig. 2). The adhesiveness of the cells from the contralateral lymph nodes (Fig. 3) did not increase significantly after sensitization ($P > 0{\cdot}05$, Student's t test).

An adhesiveness index was obtained by calculating the ratio of the adhesiveness of cells from the draining lymph nodes to the adhesiveness of cells from the non-draining lymph nodes for each time interval. Since the collision efficiency values for the non-draining lymph-node cells did not vary significantly between Day 0 and Day 92, a mean value of $12{\cdot}48 \pm 1{\cdot}21\%$ was obtained and this was used as the non-draining lymph-node-cell measurement in the adhesiveness index. Blast-cell and cellularity indices were obtained from the ratio of the draining to the non-draining lymph-node-cell values at each time interval.

Plots of draining/non-draining lymph-node indices against time after sensitization are shown in Fig. 4. As found in Figs 2 and 3, the adhesiveness index correlates more closely with the cellularity index than the lymphoblast index. Thus both the adhesion and cellularity indices remained elevated between Days 5 and 14, whereas the lymphoblast index decreased during this time.

The adhesiveness of blast lymphocyte and small lymphocyte populations

Two populations of cells were obtained by density-gradient centrifugation containing (a) 43% lymphoblasts and (b) >77% small lymphocytes (Fractions IV and VI respectively in Table II). These fractions were washed 3 times in medium and their adhesiveness was measured by Couette viscometry. As a control, unsensitized lymph-node cells were incubated in 50% Percoll (r) for the duration of the centrifugation period and these were then treated identically to the other cell populations.

TABLE III.—*The adhesiveness of blast-enriched and small lymphocyte fractions obtained by density gradient centrifugation of lymph-node cell suspensions from oxazolone-sensitized mice*

Fraction	Collision efficiency % ± s.e. mean	n	Significance
IV	$27{\cdot}5 \pm 3{\cdot}2$	6	$P < 0{\cdot}05$
VI	$21{\cdot}7 \pm 2{\cdot}0$	6	$P < 0{\cdot}05$
LNC*	$15{\cdot}8 \pm 1{\cdot}8$	6	

* LNC = normal lymph-node cells after a 30 min incubation in 50% Percoll (r) at 25°.

Although after Percoll (r) treatment the collision efficiency of the unsensitized population had risen from $12{\cdot}39\% \pm 0{\cdot}62\%$ to $15{\cdot}8 \pm 4{\cdot}1\%$ (s.e. mean, n = 6), the collision efficiencies of both the lymphoblast-enriched fraction ($27{\cdot}5 \pm 7{\cdot}3\%$) and the small-cell fraction ($21{\cdot}7 \pm 4{\cdot}6\%$) were significantly higher (Table III). Analysis by Student's t test showed that these values were not significantly different.

DISCUSSION

The mechanism by which lymphoid cells accumulate in the draining lymph nodes during certain immune responses or accumulate at chronically inflamed sites remains obscure. We have shown that during contact sensitization cell adhesiveness increases, partly owing to an increase

in the number of more adhesive lymphoblasts and partly owing to a change in the adhesiveness of small lymphocytes. The increase in the adhesiveness of the small lymphocytes may be due to direct stimulation of these cells by oxazolone, or to division of the lymphoblasts, the resulting cells being more adhesive than the initial small progenitor cell. In the latter case, the cellular process responsible for increased adhesiveness may be activated initially in both the parent and the progeny cells.

Various workers have shown a massive increase in the proliferation of thymus-derived cells in the draining lymph node during the response to oxazolone and an increase in the number of recirculating lymphocytes accumulating in the node (Asherson and Ptak, 1968; Parrot and de Sousa, 1966; Davies *et al.*, 1969; Asherson and Allwood, 1972; Asherson and Barnes, 1973). Although there is no clear evidence for immunologically specific localization of activated lymphocytes to skin allografts (Prendergast, 1964; Hall, 1967), stimulated immunoblasts do accumulate in antigenic foci, although their antigenic specificity does not seem to play an important part (Moore and Hall, 1973). Immunoblasts seem to have different migratory patterns from small lymphocytes and preferential accumulation of lymphoblasts to the mesenteric lymph nodes, Peyers patches and the lamina propria of the small intestine has been observed (Hall, Parry and Smith, 1972; Parrot and Ferguson, 1974; Olsewski and Kupiec-Weglinski, 1978).

A change in the adhesiveness of the responding cells may contribute to many of these observations. Since there is an increase in the cellularity of the draining lymph node during oxazolone sensitization, the accumulation of cells and the production of new cells must exceed their release into the circulation. If these cells are more adhesive to their surroundings in the node during this period, their entry into the circulation may be impaired, resulting in a build-up of cells in the node. This would also be consistent with the observations of "plugging" by aggregates of lymphoid cells in lymph nodes undergoing an immune response (Parrot and de Sousa, 1970; Kelly, 1970).

Changes in the adhesiveness of lymphoid cells have been associated with changes in their migratory properties *in vivo*. Glycoprotein factors produced by lymphocytes which affect their intercellular adhesiveness (Curtis and de Sousa, 1973, 1975) also affect the recirculatory properties of lymphocytes when these factors are injected into experimental animals (Davies, 1978; Curtis, 1978). Evans and Davies (1977) isolated 2 subpopulations of thymocytes with differing intercellular adhesiveness and found that these cells had different migratory patterns when injected into host animals. Since Fraction IV (43·4% lymphoblasts) is more adhesive than Fraction VI (4·6% lymphoblasts) then, even though the fractions are for technical reasons not pure preparations of one cell type, it follows that lymphoblasts must make a contribution to the elevated adhesiveness. Furthermore, since both fractions are more adhesive than control populations it would seem that small lymphocytes after oxazolone sensitization are more adhesive than small lymphocytes from non-sensitized nodes.

Our results suggesting an increased adhesiveness of lymphoblasts (Table III) support the observations of Shortman *et al.* (1972), who showed that lymphoid cells could be fractionated into subpopulations depending on their adhesion to glass bead columns and that subpopulations containing dividing cells were more adhesive than non-proliferating populations. However, the precise contribution to cell traffic of the observed increase in the adhesiveness of lymphoblasts remains to be elucidated. Nevertheless an increase in the adhesiveness of lymphoid cells during contact sensitization may be of functional importance in the immune response by altering cell movement to and from the node (*e.g.* by plug formation) and perhaps by increasing cellular interactions thought to

be necessary for optimal immunostimulation.

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