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Earliest lymphoid colonization of neonatal rat lymph nodes: an antigen-specific process?

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Summary. The present work studied the little known process of lymphoid cell colonization of neonatal lymph nodes, while considering the nodal site of entry of circulating lymphoid cells and the either random or antigen-specific character of the process. Tissue sections of a mesenteric, cervical and popliteal node from each of 57 rats, aged 4 hours to 3 weeks, were analysed. Observations bear on the relative importance of the implication of the subcapsular sinus versus venules of nodes, and the composition of their emerging lymphoid cell population by determining the proportion of lymphocytes and blast-related cells. At 16-20 hours after birth, cell counts yielded a mean proportion of 84% for blast-related cells which decreased to 18% at 3 weeks. These percentages are compatible with values expected for a selective antigen-specific entry of lymphoid cells in nodes, not with values that would result from a random entry of lymphocytes. Moreover, observations revealed that by far most colonizing cells initially enter nodes carried by the afferent lymph, little via their venules.

Key words: Lymph nodes, High endothelial venules, Lymphocyte trafficking, Immunologic surveillance, Neonates

Introduction

The initial steps in the lymphoid colonization of a new-born's lymph nodes are unclarified. Besides filling an histological gap, knowledge of this process would contribute to assess the validity of the popular view on the mode of entry of circulating lymphocytes in nodes. In 1964, a cell transfer experiment by Gowans and Knight revealed that blood lymphocytes recirculate via the nodes. The authors further suggested that circulating lymphocytes enter nodes almost only at their high endothelial venules (HEVs), and at random. Randomness was presumed to allow lymphocytes of the full repertoire of antigen-specificities to occur in each node (Rouse et al., 1984; Ager, 1994), hereby maximising the probability of encounter of an antigen sequestered in a node with rare lymphocytes competent to respond to it (Yednock and Rosen, 1989). The interest of the revealed process of recirculation clouded the questionable, but unquestioned, character of both accessory latter suggestions which nonetheless were accepted as if proven (Sainte-Marie, 1975). However, a similar but improved experiment (adding early time-intervals of observation after transfer) showed instead that a substantial fraction of blood lymphocytes enter nodes indirectly at their subcapsular sinus: carried by the afferent lymph (Sainte-Marie et al., 1975). Recently, a comprehensive analysis of the state of nodes and HEVs under various immunological conditions further supported this conclusion; in the case of each condition moreover, it revealed an antigen-specific entry of lymphocytes in nodes (Sainte-Marie and Peng, 1996). Ponzio and Thorbecke (1988) explained that a lack of a direct proof for such expectably selective process is due to experimental difficulties.

In view of this, I consider that the knowledge of the appearance and ontogeny of the lymphoid cell population in neonatal nodes, which remains unclarified to date, could support either of the above opposite opinions. At birth indeed, nodes are virtually devoid of lymphoid cells. In addition, only naïve lymphocytes exist then and solely a trivial fraction of them is competent to respond to a given antigen (Ager, 1994; Goodman, 1994). The appearance in nodes of blastogenic cells (intermediate between an activated lymphocyte and a blast) and blasts (immunoblasts) occurs after rare competent lymphocytes are postnatally induced to undergo blastogenesis by the proper antigen. Since an also tiny fraction of the repertoire of antigens challenges a node, soon after birth the percentage of nodal blast-related cells (further including prelymphocytic cells derived from blasts) should consequently be low if circulating lymphocytes enter nodes randomly, but very high if the entry is antigen-specific. Present data favor the latter alternative.

Materials and methods

Pregnant 3-month-old Sprague-Dawley rats were obtained from Charles River; their progenies appeared

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healthy. Groups of three neonates of either sex were sacrificed at 4, 8, 12, 16, 20 and 24 hours after birth; thereafter, groups were sacrificed every 6 hours up to 48 hours, and every second day from day 3 to 15 as well as on day 18 and 21. From each of the 57 neonates, nodes of the superficial cervical, mesenteric (along with samples of the small gut until day 3), and popliteal sites were removed. Seven nodes, mostly of the popliteal site, were not obtained mainly because of their minute size. The tissues were fixed for 24 hours in a solution of Bouin-Hollande. The latter is a solution of Bouin with neutral cupric acetate, to which acetic and trichloracetic acid are added just before use (Langeron, 1949). Paraffin-embedded nodes were cut serially at 7 μm. All sections were mounted in the case of animals younger than 7 days, and every fourth section in the case of older ones. Sections were stained with the M-G-Giemsa technique (Langeron, 1949).

Cell counts and data analysis

Cells of each of three categories (1: lymphocytes, 2: blastogenic and prelymphocytic cells, 3: blasts) were counted in a cervical, mesenteric and popliteal node from each neonate. In each of the 164 analysed nodes, cells were counted within a standard surface area (square with 220 μm sides), determined using a divided grid in one eyepiece of the microscope at x320. The number of areas analysed per node was 5 in rats younger and 3 in rats older than age 48 hours for mesenteric nodes and than age 72 hours for popliteal or cervical nodes. The areas were taken randomly wherever lymphoid cells occurred in the cortex of the little developed nodes of the neonates <48 hours old. In the case of the more developed nodes of neonates >72 hours old, one area was taken randomly in each of the three following sites: the peripheral cortex, the center of a deep cortex unit, and the corticomedullary junction of each node. This last site comprised areas where either peripheral or deep cortex was contiguous to the medulla. Relative abundance of each cell category was calculated as the quotient of count for one category over count for all categories, multiplied by 100. Temporal trends in relative abundance of each category were analysed using Spearman's rank correlation (Sokal and Rohlf, 1981).

Results

All lymphoid cells stained blue to violet. Lymphocytes had a very thin rim of pale blue cytoplasm around a small and dark nucleus. Blasts were the largest of the lymphoid cells. Their cytoplasm was the most abundant and most basophilic, while their nucleus was the largest and had the most prominent nucleolar apparatus. Blastogenic and prelymphocytic cells showed various morphological intermediate forms between a lymphocyte and a blast, or a blast and its progeny lymphocytes, respectively. It was not necessary to attempt to distinguish these cells into B and T cells. Indeed, it is known that during the first week after birth (day 0-8), when colonization takes place, nodal lymphocytic cells are almost exclusively T cells (Eikelenboom et al., 1979).

Throughout the period of investigation, we commonly observed variably marked differences between the timing and/or the degree of development of the various lymphoid populations, the particular structures sheltering them as well as the HEVs which emerged in different portions of a same node. Such developmental differences also occurred among nodes at different sites in a same animal and among corresponding nodes from individuals of a same age. The mesenteric nodes were the most developed at any given time. Their morphology will be reported in neonates aged 4-24 hours and about 1, 2 and 3 weeks.

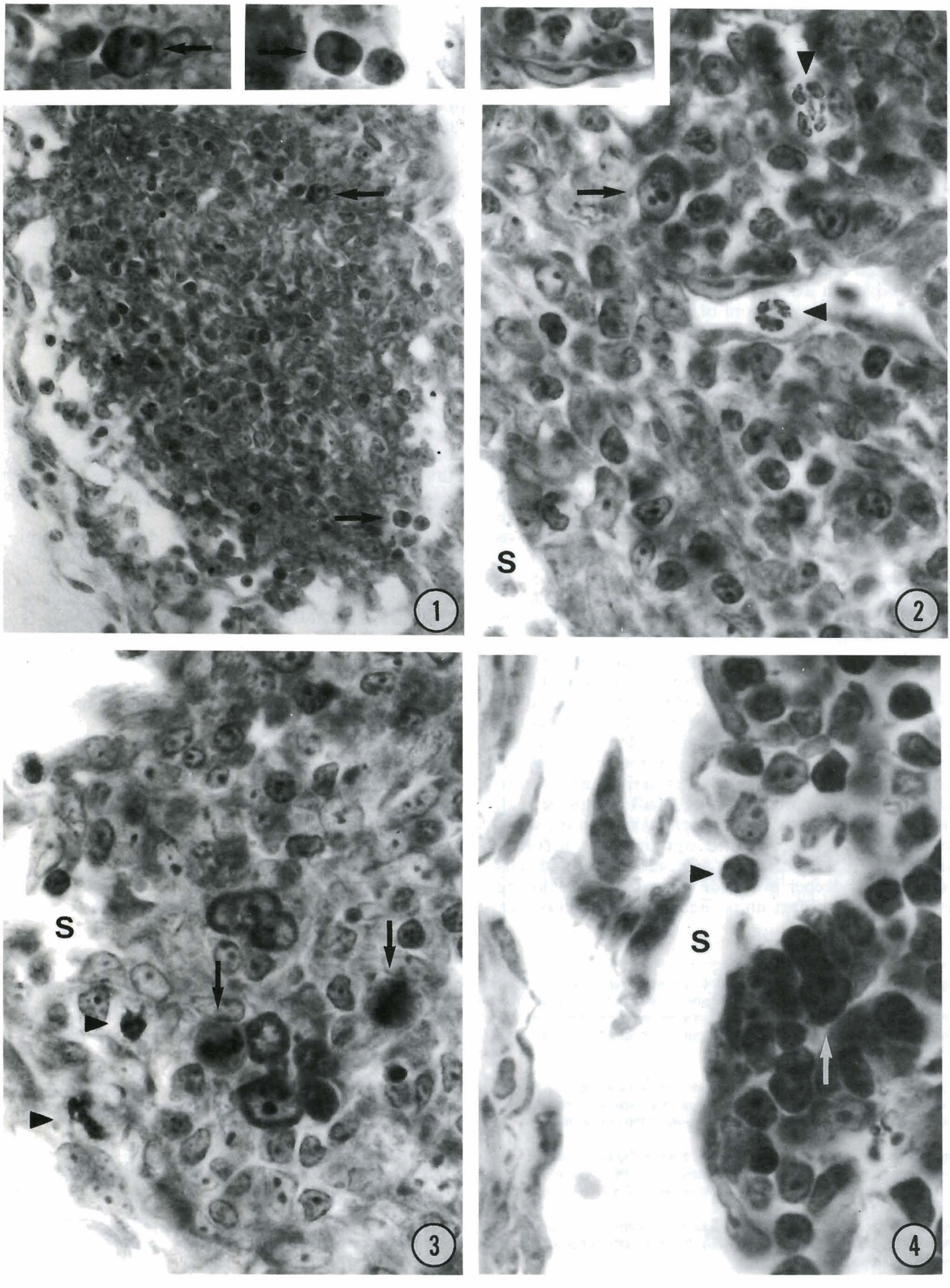
Let us point out that the term "unit", used in our paper, always refers to a "deep cortex unit". Each of the usually several units of a node represents a distinct portion of the deep cortex (so-called paracortex). A unit occurs in topographical relationship with each opening of afferent lymphatics into the subcapsular sinus of a node (Belisle and Sainte-Marie, 1981a,b). A developed unit is roughly hemispherical: it adheres by its flat face to a portion of above peripheral cortex and its curved face bulges into the overall medullary area beneath. Each unit comprises a "center" (unit center) and a "periphery" (unit periphery). The periphery is a thick layer which covers the curved face of the unit center: isolating the unit center from the medulla. Note that tissue organization of neonatal nodes having been previously described and illustrated (Bélisle and Sainte-

Fig. 1. Four-hour-node. A late blastogenic or blast cell (lower arrow), enlarged in the right inset, adheres to the inner wall of the pale subcapsular sinus at right. In such a neonatal node, tissue organization is not yet well defined and the sinus' inner wall is not clearly outlined. A similar cell (upper arrow), enlarged in the left inset, is present in the cortex. As usual in new-borns, the lymphocyte-devoid cortex consists mostly of poorly outlined reticular-like cells. x 350

Fig. 2. Four-hour-node. A blast or late blastogenic cell (arrow) occurs in the cortex. Arrowheads point to neutrophils: one located in the cortical parenchyma, another in the lumen of a HEV precursor developing near the pale subcapsular sinus (S). Inset enlarges a lightly thickened endothelial cell of this vessel showing a basophilic cytoplasm at right. x 900

Fig. 3. Eight-hour-node. Two mitotic blasts (arrows), present in a group of blast-related cells located close to the pale subcapsular sinus (S), exhibit a typically well-outlined and basophilic cytoplasm unlike mitotic reticular-like cells with pale cytoplasm (arrowheads). x 900

Fig. 4. Twelve-hour-node. The light connective tissue capsule is at extreme left along the colorless subcapsular sinus (S). The arrowhead points to a sinusal lymphocyte. An elongated blast (arrow) appears as migrating near or across the inner wall of the sinus. X 900.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Marie, 1981b), repetitions will be avoided or restricted here.

Nodes of 4 to 24-hour-old neonates

At 4 hours, a cortex and a medulla were distinguishable in each node. The cortex underlaid the subcapsular sinus. Its reticular fiber meshwork comprised numerous large pale and ill-outlined reticular-like cells but rare darker lymphoid cells. The medulla laid between the cortex and a node's hilus. It typically consisted in an alternance of cords and lymphatic sinuses, both being devoid of lymphoid cells. At 24 hours, the lymphoid population of the cortex had increased but it was sparse, except for some small rounded areas with a denser population which developed in the deeper stratum of the cortex. In places, lymphoid cells were almost restricted to such areas which represented the emerging "centers" of the units of deep cortex: each arising in topographical relationship with the openings of afferent lymphatics.

The lymphoid cell population comprised lymphocytes and blast-related cells. A few blasts were seen as early as 4 hours (Figs. 1, 2); scarce mitotic blasts were first encountered at 8 hours (Fig. 3). These various cells occurred in the subcapsular sinus and the cortex; and in places, a blast crossed this sinus' cortical (inner) wall (Fig. 4). From 8 hours on, blast-related cells increased in number in the subcapsular sinus, where they generally were in contact with its cortical wall (Figs. 5-8). Under favorable cutting incidences showing the openings of afferent lymphatic vessels into this sinus, these cells were moreover observed to be generally located just beneath such openings (Fig. 5). Overall however, most lymphoid cells occurred in the cortex. There, they were initially present mainly next to the subcapsular sinus, often forming small groups beneath sites of adherence of sinusal lymphoid cells to the sinus' cortical wall: i.e. beneath openings of afferent lymphatics (Figs. 9-12). With time, most lymphoid cells were situated deeper in a node towards the developing centers of deep cortex units. Here and there moreover, small concentrations of blast-related cells formed "trails" stretching into the cortex from the subcapsular sinus. Lastly, it was noticed that, as throughout the studied 21-day-period, sinusal blasts appeared to be more abundant in the mesenteric nodes than in other nodes.

No typical HEVs occurred at 4 hours. But, a few endothelial cells had thickened slightly in some precursor standard venules (Fig. 2). Unlike in mature HEVs with characteristic thick endothelial cells, just rare blood lymphocytes were bound to the endothelium of these HEV precursors; lymphocytes crossing their walls were correspondingly rare. In another respect, the lamina propria of intestinal villi exhibited scattered lymphocytes at the same 4-hour-term.

Nodes of about one-week-old neonates

The peripheral cortex as well as the deep cortex units were better differentiated. The overall lymphoid population of the peripheral cortex had a greater density than earlier. However, in many places of a node, lymphoid cells were often still almost limited to deep cortex units.

The deep cortex units had developed typical features. Hence, each unit had a grossly hemispherical shape protruding into the medullary area. Moreover, each unit now had a "periphery" in addition to its "center". The unit centers generally remained more densely populated with lymphoid cells than the unit peripheries and the peripheral cortex. As a distinctive feature of neonatal unit centers, their concentration of resting and mitotic blasts was markedly greater than in mature nodes.

Developing HEVs occurred in the peripheral cortex and the peripheries of the deep cortex units. Their endothelial cells were slightly to moderately thickened, and still showed rather few endothelium-bound or diapedesing lymphocytes.

Nodes of about two-week-old neonates

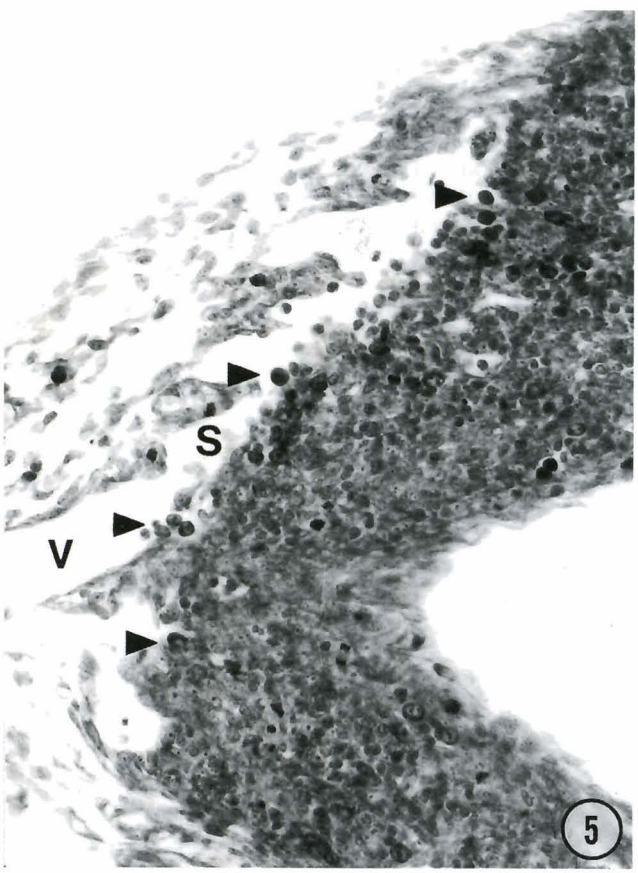
Rounded "follicles" had appeared in the peripheral

Fig. 5. Eight-hour-node. V indicates the opening of an afferent lymphatic vessel into the also colorless subcapsular sinus (S). Blasts or blastogenic cells, pointed by the second lower arrowhead, adhere to the sinus' inner wall just beneath the opening (see Figs. 6 and 7). Comparable sites of similar adhering cells are indicated by other arrowheads: consecutive serial sections showed that each such a site occurred beneath an afferent lymphatic opening. In the relatively light cortex, populated mostly by pale reticular-like cells with sparse lymphoid cells, dark blast-related cells are present mainly at proximity of the sinus. x 220

Fig. 6. Enlargement from Figure 5. The arrowhead corresponds to the second lower arrowhead in Figure 5. The upper arrow points to blastogenic or blasts cells accumulated beneath the sinus' inner wall at a site where lays such a sinusal cell (indicated by second upper arrowhead in Figure 5). The lower arrow points to similar blast-related cells in the cortex. Note that the medulla is not seen in this section of the node. x 350

Fig. 7. Photomontage of enlargements of sites in Figure 5, each taken at a slightly different focus to compensate waving of tissue section. The upper arrow indicates an accumulation of blastogenic cells or blasts located above or beneath the sinus' inner wall. The lower arrow indicates two such sinusal cells laying on the sinus' wall and shown in Figure 5 by second lower arrowhead. x 800

Fig. 8. Enlargement of the site (rotated at 90°) pointed by the uppermost arrowhead in Figure 5. V indicates the opening of an afferent lymphatic vessel into the colorless subcapsular sinus (S). Several cortical blastogenic or blast cells appear as if migrating from this sinus across the thickness of the relatively light cortex. x 400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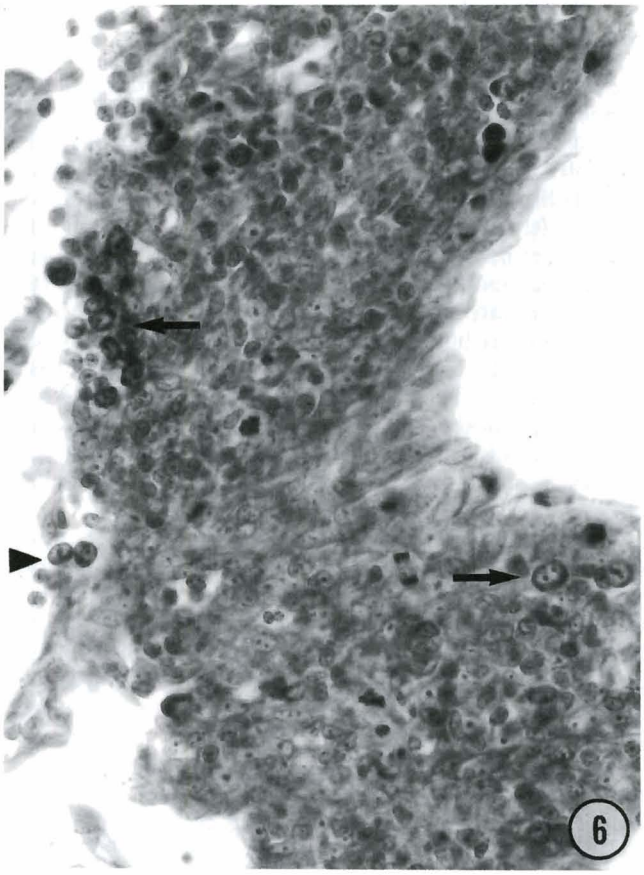
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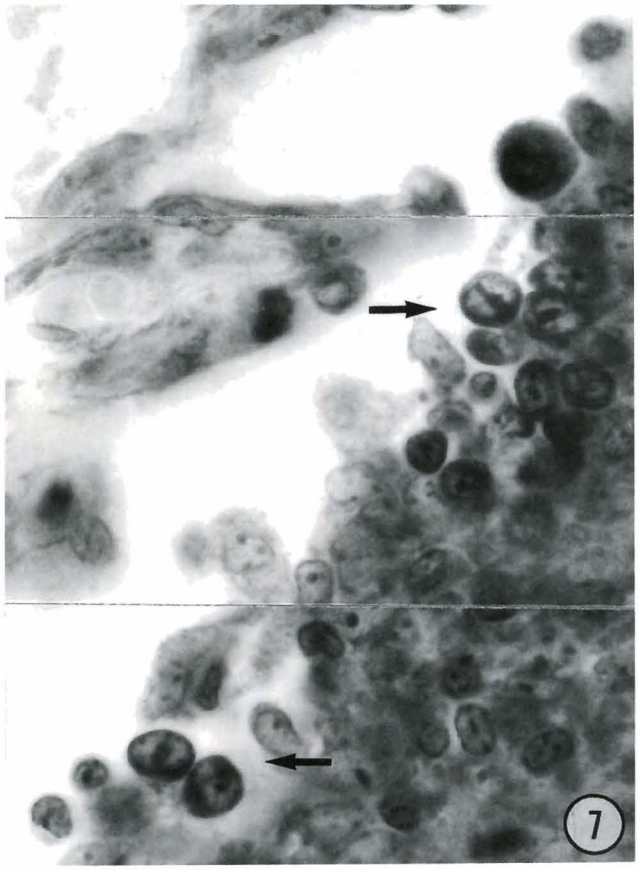
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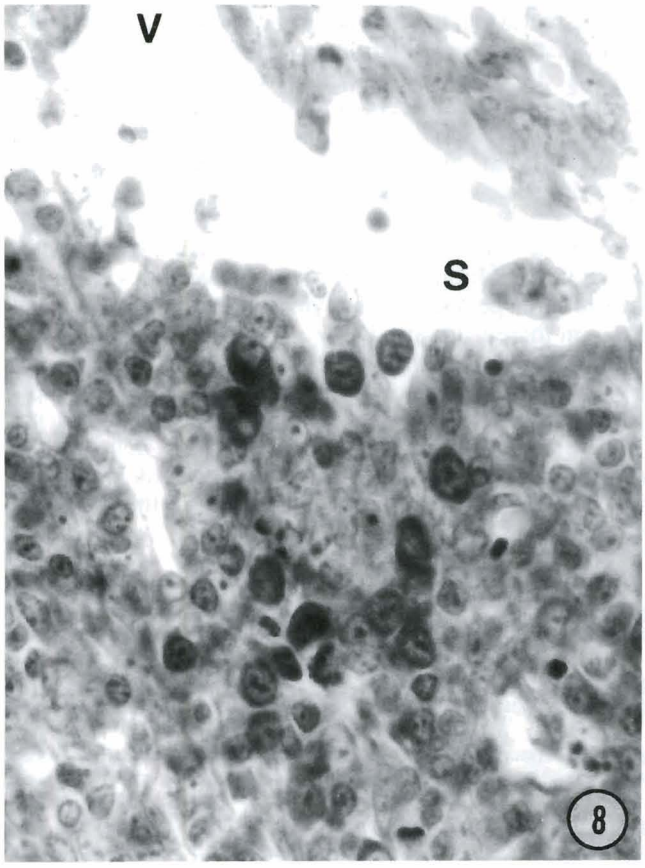
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cortex. They formed a discontinuous row under the subcapsular sinus, each being separated from the next one by an area of the earlier peripheral cortex termed an "extrafollicular zone" Whereas most of these recently formed follicles hosted lymphocytes only, already a few further sheltered an emerging tiny islet of cells typical of a nodule (germinal center). The extrafollicular zone, like the peripheries of the deep cortex units, was more densely populated by lymphoid cells than the earlier peripheral cortex but still not as much as the unit centers. From day 7 to 13, moreover, a peculiar phenomenon was noted in one or a few deep cortex units of some nodes: the little populated periphery of such a unit hosted a very high concentration of blast-related cells, accompanied by much fewer lymphocytes, than the periphery of the other units in a same node.

As the population of lymphoid cells in the nodes increased between day 7 and 13, blasts-related cells appeared to be in a lesser concentration in the cortex than earlier but nonetheless remained more concentrated than in mature nodes (Figs. 13, 14). In a few places moreover, there still occurred a "trail" of blast-related cells extending from the subcapsular sinus into the cortex (Figs. 15, 16).

Like in mature nodes, the developing HEVs were restricted to the extrafollicular zone and the unit peripheries, each unit periphery being continuous with the overlying area of extrafollicular zone. Incidentally, both similarly-constituted latter cortical components form the pathways for intranodal migration of incoming cells from the subcapsular sinus or HEVs (Sainte-Marie and Peng, 1996). Many HEVs had acquired some much thickened endothelial cells and exhibited endothelium-bound and diapedesing lymphocytes more frequently than a week earlier. In the unit peripheries moreover, narrow lymphatic sinuses (unit sinuses) had arisen as culs-de-sac extending from the cortical extremities of medullary sinuses. Lymphocytes were present in these unit sinuses. Medullary sinuses contained variable numbers of dispersed lymphocytes, and occasional blasts were seen there from day 18.

A blast concentration occurred in portions of medullary cords contiguous to the periphery of deep cortex units: its importance in a given cord matched that in the related area of the unit periphery. Relatively numerous plasmacytes, and intermediate cells between blasts and plasmacytes, were also present in most of the same cords. By contrast, such cells were rare, or absent, in the cords not directly related to a unit periphery but instead contacting areas of peripheral cortex present in between spaced deep cortex units. Incidentally, plasmacytes were occasionally detected as early as day 5, but in small numbers and in just a few cords of some cervical or mesenteric nodes.

Nodes of about three-week-old neonates

The nodal morphology overall resembled that of mature organs. Thus, all follicles now appeared to shelter a nodule (germinal center). Concentration of blast-related cells had decreased in the extrafollicular zone of the peripheral and in the deep cortex. Moreover, whereas blast-related cells had earlier accounted for a large fraction of the lymphoid cells in the subcapular sinus, their relative importance had waned there also. Nonetheless, some blasts again occurred within, as well as just beneath, this sinus. Lymphocytes were now the predominant lymphoid cells in this sinus as in the cortex. In places, lymphocytes could be abundant at the opening of an afferent lymphatic into the subcapsular sinus and in the neighboring sinusal area. Actually, the latter phenomenon could as well be at time observed in an occasional node of a younger animal (Fig. 15) which illustrates above mentioned possible marked differences in the timing of a given degree of development of a lymphoid population in different nodes or animals.

HEVs were variably developed depending on location in a node. Some had typically high endothelial cells and showed numerous endothelium-bound and diapedesing lymphocytes. Let us point out that, at all time-intervals of this study and apart from polynuclear neutrophils, virtually only lymphocytes (i.e. no blast-related cells) were observed in the blood of nodes or bound to the endothelium of venules. Lastly, most medullary cords had become populated by plasmacytic cells: the most immature forms of this cell type remained situated next to the corticomedullary junction, the most mature forms being located close to a node's hilus.

Cell counts

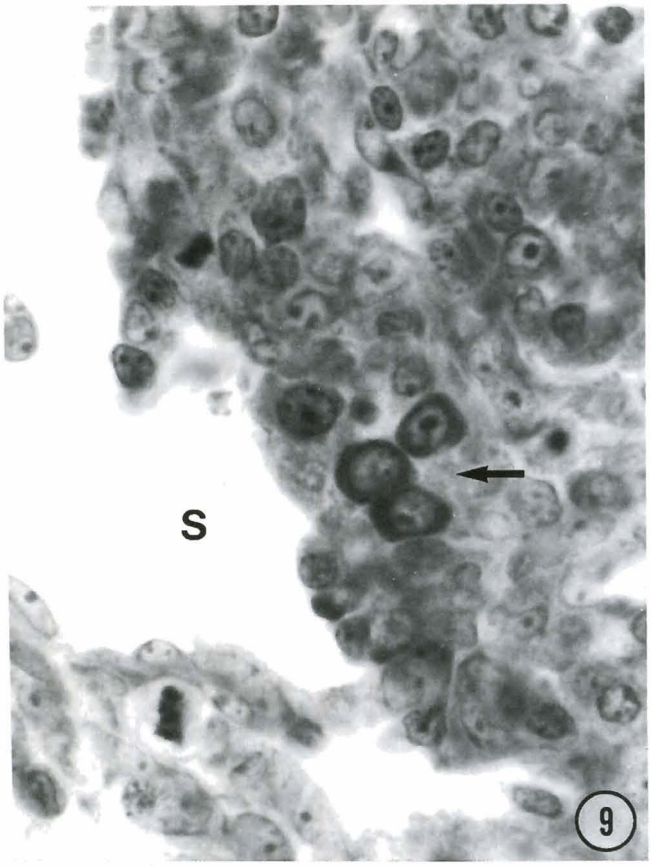
The absolute number of lymphoid cells per surface area was highly variable, depending on age, anatomical site and/or location of an area analyzed within a given

Fig. 9. Eight-hour-node. A few blasts-related cells (arrow) are present in the outermost layer of the cortex just beneath the inner wall of the subcapsular sinus (S). Tridimensionally, such cells are there more numerous than it seems in this single micrograph as shown by Figure 10. x 900

Fig. 10. Same tissue section as for Figure 9 but taken at a slightly different focus. Two additional blast-related cells (arrowhead), adhering to the inner wall of the subcapsular sinus, are detected here. Another blast-related cell (arrow), located deeper in the cortex, is also better seen here. x 900

Fig. 11. Eight-hour-node. A late blastogenic or blast cell (arrow) adheres to the inner wall of the pale subcapsular sinus devoid of lymphocytes. Three other such cells occur at left in the cortex, just beneath the sinus inner wall. x 900

Fig. 12. Eight-hour-node. A compressed late blastogenic or blast cell (arrow) is probably located in the collapsed subcapsular sinus. Several such cells occur close to it; at least most of them are present in the outermost layer of the relatively light and sparsely lymphocyte-populated cortex. x 900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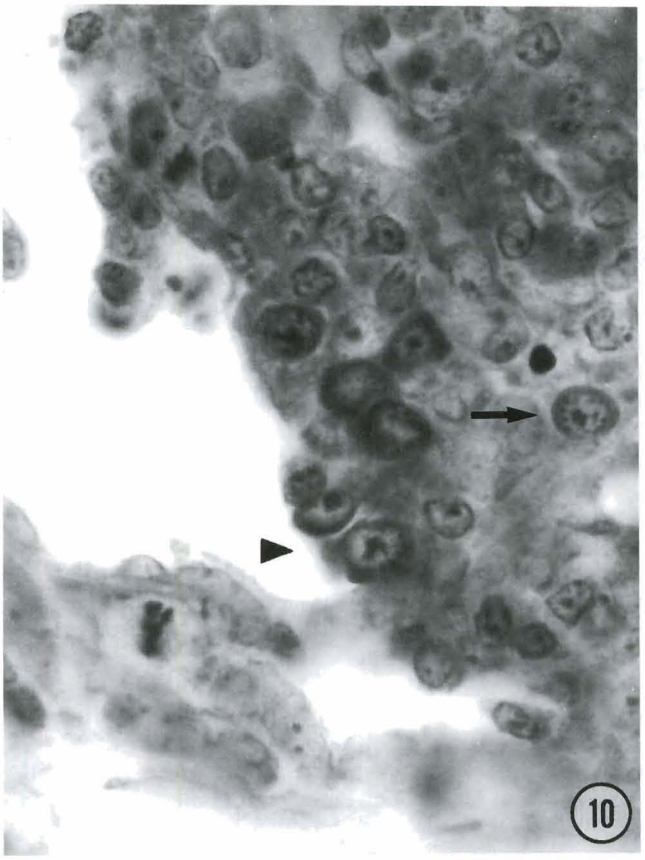
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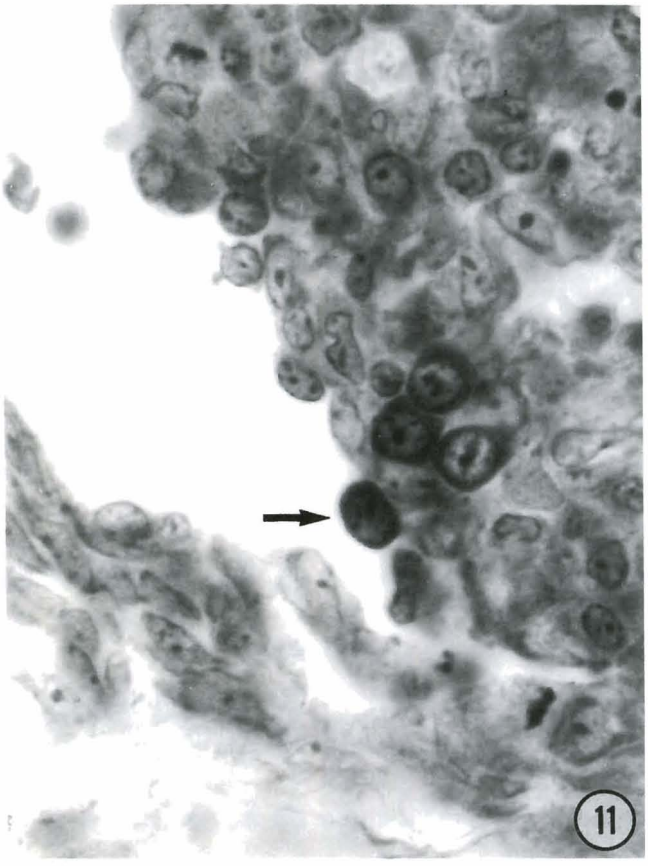
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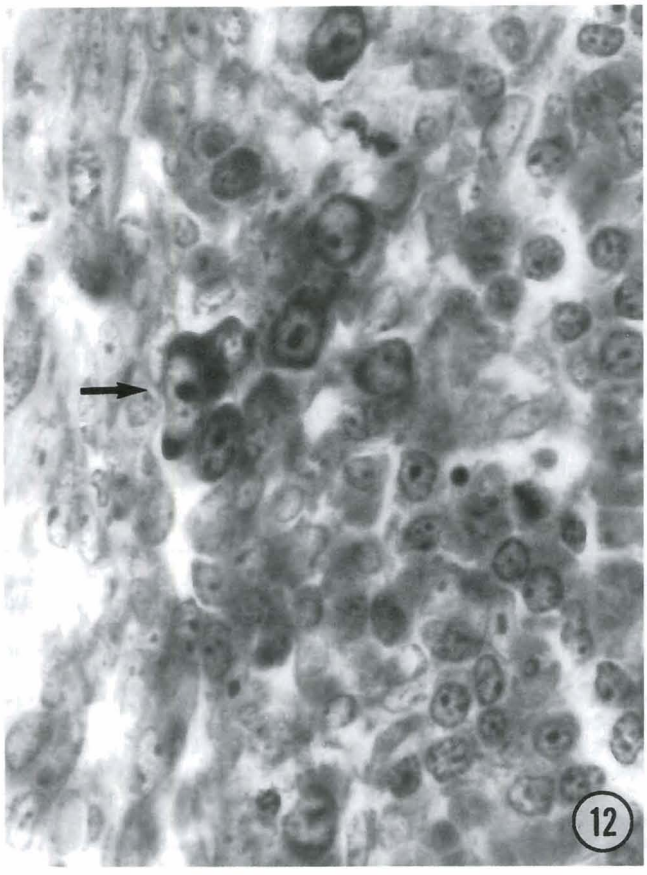
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component of a node; this number varied from a few to 463 cells. Variation also occurred in the relative numbers of cells of each of the three categories among nodes of a same age and a same anatomical site (see standard deviations in Figure 17).

Temporal variation in proportion of cells of the three categories is shown in Figure 17. In nodes from the three sites, there was a highly significant correlation between relative abundance of blast-related cells and time elapsed since birth, their representation decreasing from around 86% at 4 hours to 18% on day 21. Conversely, the proportion of lymphocytes increased with time elapsed since birth. Note that counts included neither the plasmacytic cells of the medulla, nor the typical cells of the nodules (germinal centers), mentioned above.

Discussion

Timing of development of the lymphoid cell populations of nodes is species-dependent. In the sheep, development starts prenatally (Morris and Simpson-Morgan, 1985). In most studied species, including the rat (Eikelenboom et al., 1979), it normally occurs postnatally upon exposure of a new-born to antigens. Even in this group of species however, an unusual intrauterine infection can initiate a precocious lymphoid development of fetal nodes (Silverstein and Lukes, 1962; Esterly and Oppenheimer, 1969; Singer et al., 1969). The present study is based on the normal rat, as was the work on lymphocyte recirculation by Gowans and Knight (1964). A popular view is that fetal nodes, virtually devoid of lymphoid cells, are colonized just prior to or after birth by blood lymphocytes formed in the fetal thymus and that enter nodes randomly at HEVs (Harris and Ford, 1963; Miller, 1966; Murray and Murray, 1964; Töró and Oláh, 1967). Our study reveals instead that neonatal nodes are initially colonized by mostly lymph-carried blast-related cells which enter their subcapsular sinus together with fewer lymphocytes. These facts had not been reported before, likely because previous studies were not concerned with the problem considered here. In these studies moreover, nodes were examined just at birth or several days later (Söderström, 1967; Eikelenboom et al., 1978) whereas the most revealing events unfold during the few first days and particularly the first 16-20 hours.

Note that our data reveal simply the general trend in the development of the lymphoid population in neonatal nodes. Exact timing and amplitude of development differed in various compartments of a same node. Incidentally, "compartments" are morphological and functional subdivisions of a node: each related to the opening of a distinct afferent lymphatic vessel and influenced only by its particular content (Sainte-Marie et al., 1975, 1982). Timing and amplitude of development similarly differed in various nodes of a same animal, as in corresponding nodes of animals of same age. The differences probably reflect the uneven or asynchronous influence of factors such as: the exact times or sites of the penetration of antigens into a neonate, the individual variation in the importance of the contamination of a new-born, the level of maternal contribution to the immunological defense of a new-born and the intrinsic immunological potential of individual neonates. Moreover, an occasional particular condition may activate naïve cells prenatally (Kyriazis and Esterly, 1970), probably accounting for the observed precocious presence in some nodes of blasts at 4 hours or few plasmacytes at day 5.

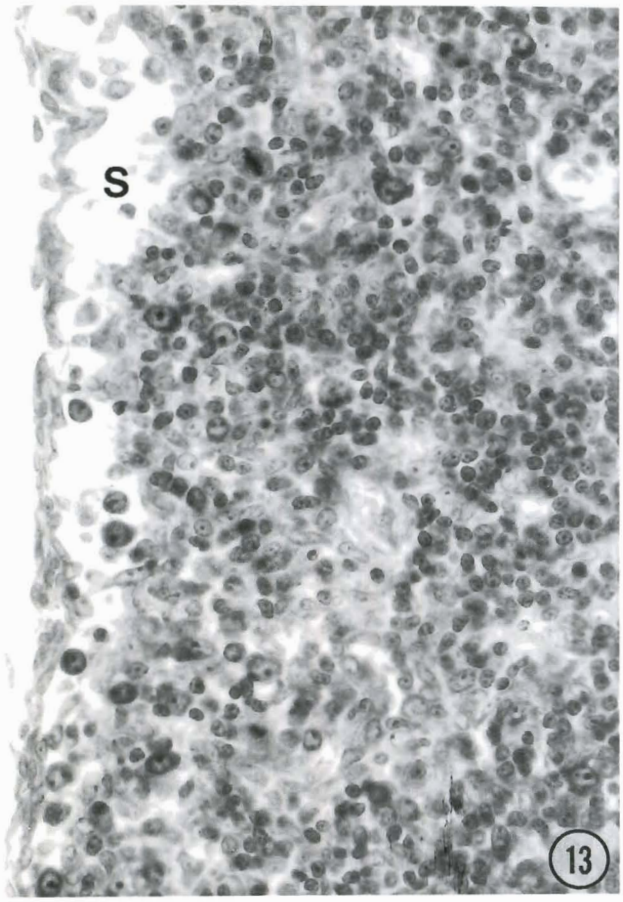
An obvious conclusion from our work is that early colonizing lymphoid cells enter nodes at the subcapsular sinus, and rarely at venules. The early relative abundance of such cells in this sinus, together with a simultaneous scarcity of lymphocytes crossing venules' walls and an absence of typical HEVs, indeed betray that the peripheral lymph is initially the principal provider of lymphoid cells. Actually, lymphocytes were previously observed in the subcapsular sinus, one minute after birth (Bélisle and Sainte-Marie, 1981a,b). These facts, in conjunction with a relative abundance of blast-related cells in the cortex next to sites of adherence of such cells to the subcapsular sinus' cortical wall and their concomitant absence in medullary sinuses, further mean that lymph-carried cells did colonize the cortical parenchyma of nodes. In line with this, we did not observe accumulations of lymphoid cells next to precursor forms of HEVs which could have suggested a direct entry of blood cells there. This was particularly obvious in the case of occasional unit peripheries with a very high proportion of blast-related cells and a scarcity of lymphocytes. Since these unit peripheries also had emerging HEVs, they should have shelter a high

Fig. 13. Nine-day-node. Several blastogenic or blast cells occur in the subcapsular sinus (S) almost devoid of lymphocytes. Many other such cells occur in the adjacent and still poorly lymphoid-populated outermost layer of the peripheral cortex. x 350

Fig. 14. Nine-day-node. Numerous blastogenic or blast cells are present in an area of cortex also much lymphocyte-devoid. x 350

Fig. 15. Thirteen-day-node. Left arrow points to the lymphocyte-rich opening of an afferent lymphatic into the light subcapsular sinus. Lymphocytes are further present in a portion of the sinus on either side of the opening. The dark cortex is now densely populated, mostly by lymphocytes. The medulla is the light and loosely populated area along right side of Figure. The right arrow indicates a "trail" of blast-related cells stretching from the subcapsular sinus; many of these appear accumulated close to the corticomedullary junction (see Fig. 16). x 50

Fig. 16. Enlargement from Figure 15 (re-oriented at 90°). The blast-related cells pointed to in Figure 15 appear as if coming from a restricted site of the pale subcapsular sinus (S). A mitotic blast (arrow) occurs near the sinus. x 400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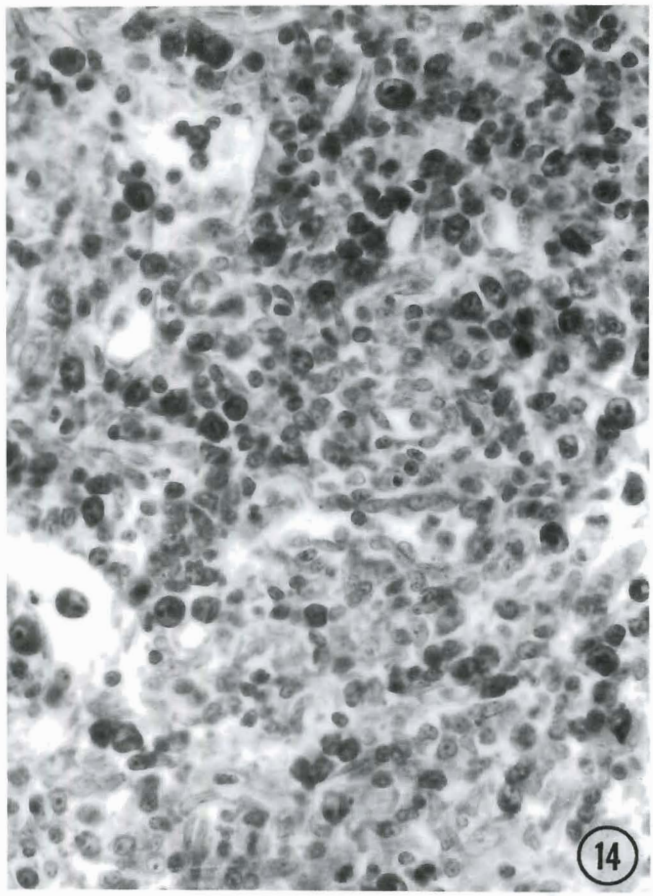
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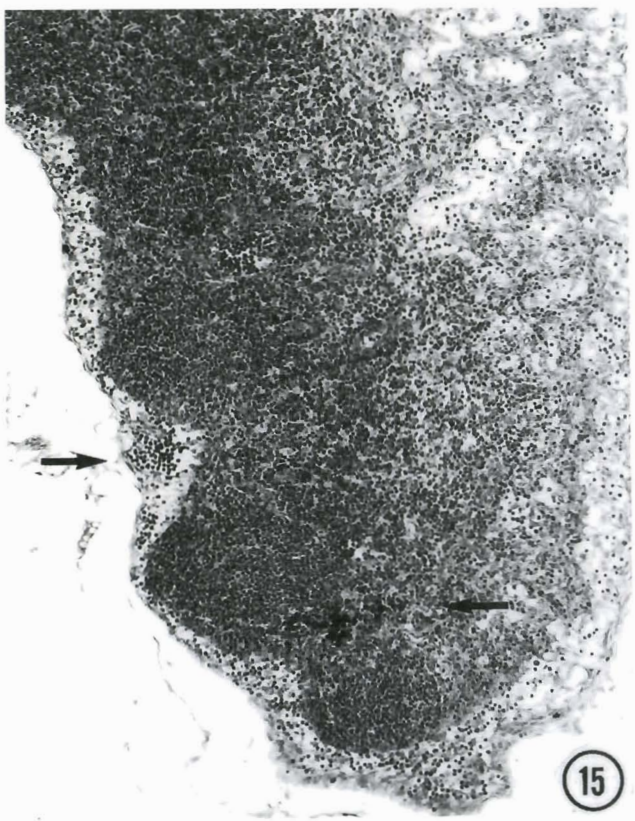
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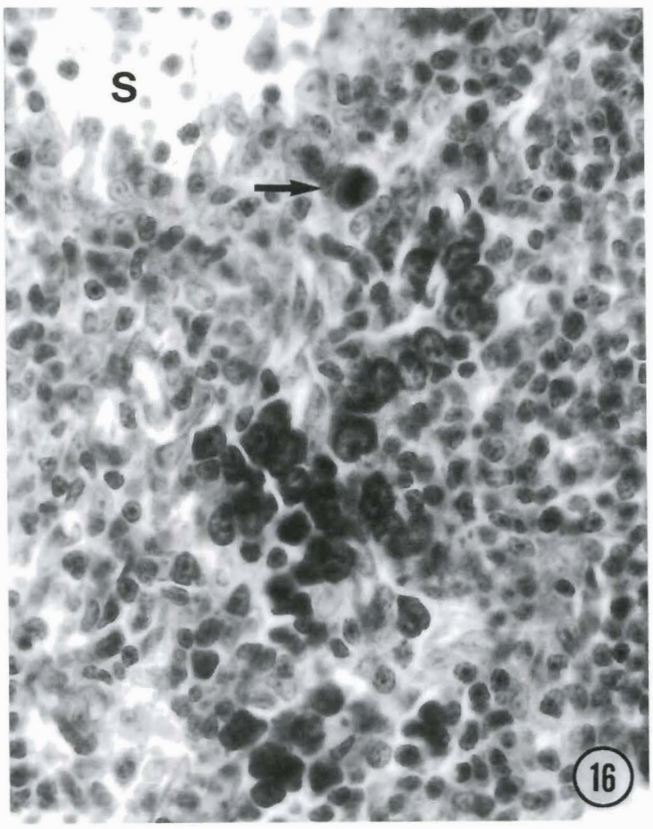
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percentage of lymphocytes like the other unit peripheries, if blood lymphocytes would directly enter there at random, which was not the case. Therefore, colonization by lymph-carried cells appeared to precede the emergence of typical HEVs. Hence, the afferent lymph being by far the main provider of earliest nodal lymphoid cells, the role of HEVs as additional sites of entrance of circulating lymphocytes arises secondarily: then, they supplement the cell contribution of the afferent lymph which reinforces ongoing local immune responses (Sainte-Marie and Peng, 1996).

A second conclusion is that colonization appears to be an antigen-specific process, and not due to a random or unselective entry of blood lymphocytes in nodes as is generally contended. The latter opinion originated from a statement by Gowans and Knight (1964) that transfused lymphocytes appeared to home in equal concentration to the various nodes of recipients which suggested to them that lymphocytes show no predilection for particular nodes. However, the statement rested on a cursory observation that was never supported by appropriate investigation and data. And what is more, the authors even reported in the same paper that a "segment" (undefined) of a given node might contain a large number of transfused cells while an adjacent segment contained only a few. This fact raises doubt about the purported uniform concentration of transfused cells, and it is little reconciliable with an unselective homing. In the present study, the importance of the entry of lymphoid cells in a new-born's node (i.e. the degree of development of its lymphoid population) varied markedly with the site of a given node and among compartments of a same node, which indicates a selective process. In accordance with this, let us recall that in athymic nude rats and mice, as well as in N:NIH (S) II nu/nu mice further deleted of B cells, a minority of

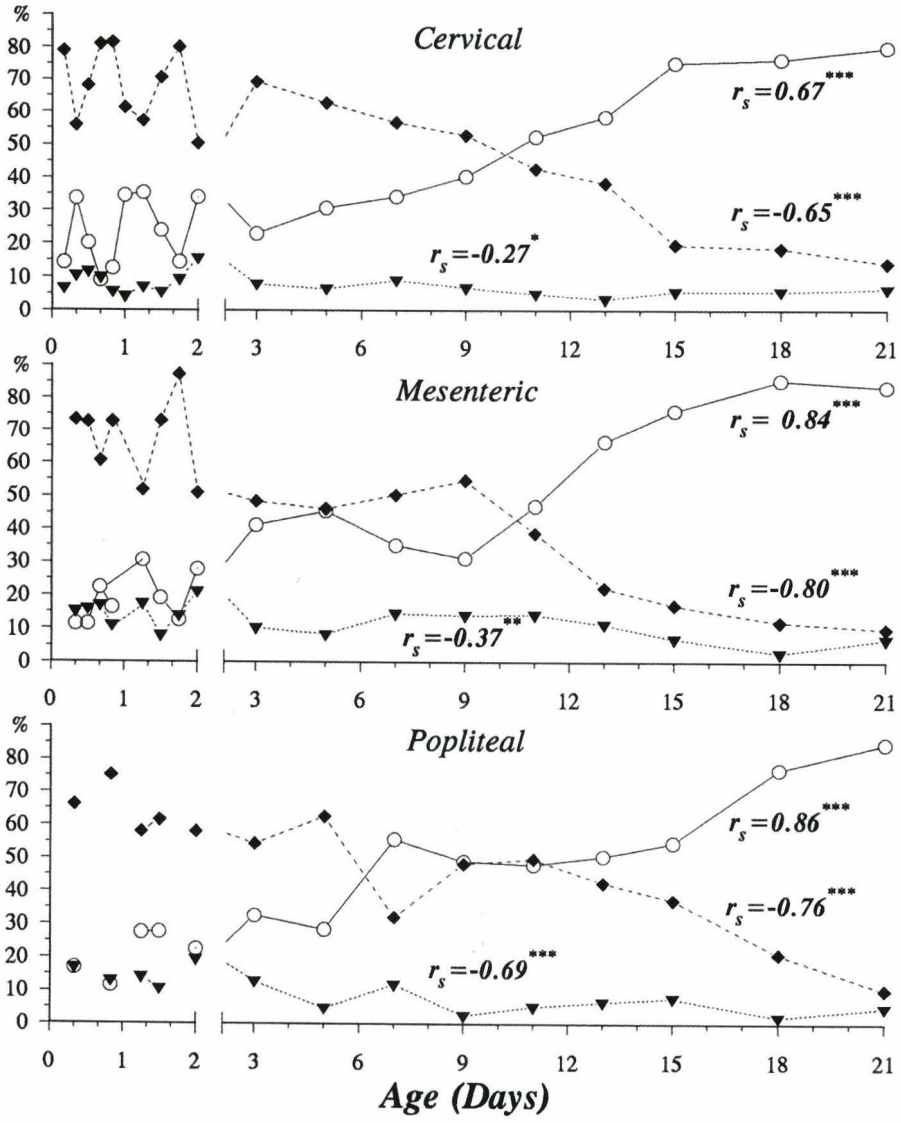

Fig. 17. Mean relative abundance of lymphocytes (circles), blastogenic or prelymphocytic cells (squares) and blasts (triangles) present in cervical, mesenteric or popliteal nodes of neonatal rats, aged 4 hours to 21 days. Spearman correlation coefficients (rs) were calculated on raw data and are all significant *: p<0.05; **: p<0.01; ***: p<0.001.

cervical nodes have exceptionally very well developed populations of lymphoid and plasmacytic cells unlike the other nodes of same animals. This too means a selective colonization (Sainte-Marie and Peng, 1983, 1984, 1985).

In further support of our latter conclusion, it is known that a given lymphocyte becomes a blast upon activation by the proper antigen: a specific process comprising the transient existence of various intermediate forms of non-proliferative blastogenic cells. In this regard, the most pertinent of the present data are those obtained for the first 16-20 hour period after birth. This period permits earliest activated naïve cells to become successively blastogenic and blast cells, but is not sufficiently long to allow the blasts to give rise to prelymphocytic cells and lymphocytes of immune responses (Stites et al., 1994). Since only a trivial fraction of the mosaic of antigens challenges a node, the percentage of nodal blastogenic cells and blasts should be quite low during this period if entry of circulating lymphocytes in a node is at random and involves naïve cells of the full repertoire of antigen-specificities. At best, only one out of every 100,000 circulating lymphocytes is competent to respond to a given antigen (Ager, 1994): i.e. 0.001%. In fact, the mean percentages of blastogenic cells and blasts reached 71% and 13% respectively, at 16-20 hours. These values and especially their sum (84%) are much too high to be compatible with a random lymphoid colonization of neonatal nodes. They are instead consistent with an antigen-specific process whereby a vast majority of colonizing cells undergo blastogenesis. In line with this, let us recall that following the atrophy of a compartment in a node of an immunodeficient adult animal, a compensatory "compartment replica" can form on the outer wall of the subcapsular sinus: opposite to the atrophied compartment and next to the opening of its related afferent lymphatic. A replica arises as an intracapsular islet of lymphoid cells also with a high concentration of blast-related cells (Sainte-Marie and Peng, 1987). Such late de novo formation of a compartment thus mimics the neonatal ontogeny of a nodal lymphoid cell population as observed here; it therefore supports the same interpretation.

Changes in the proportion of cell types after 20 hours are more complex to interpret because of asynchronism in the blastogenesis of successively activated lymphocytes so that only a global, but quite likely, explanation can be offered. Starting about 24-48 hours after birth, an early formed blast gives rise to progressively maturing proliferative and transient prelymphocytic cells: cell forms intermediate between a blast and lymphocytes of cellular immune responses. Indeed, lymphoid cells entering rat nodes during the "first week" after birth are almost all T cells (Eikelenboom et al., 1979), as is the case in mice (Raff and Owen, 1971; Chanana et al., 1973). Moreover, they accumulate in arising centers of deep cortex units (Bélisle and Sainte-Marie, 1981), i.e. in T areas of development of cellular immune responses (Oort and Turk, 1965; Sainte-Marie et al., 1990). With standard histology, prelymphocytic cells are hardly distinguishable from blastogenic cells so that the percentage of each of these two forms of precursor cells could not be assessed. Nonetheless, each of these two percentages is obviously lower than that of all combined forms of precursor cells (blastogenic, blast and prelymphocytic cells) of lymphocytes. Whatever the case, the proportion of combined forms of precursor cells decreased progressively from 84% at 20 hours to 18% on day 21. But even the latter value is not reconciliable with the much lower percentage of precursor cells that would expectedly result from a very great dilution by abundant randomly incoming unactivated naïve cells. Indeed, at most only 0.001% of circulating lymphocytes are competent to respond by blastogenesis to a given antigen (Ager, 1994) while only a tiny fraction of the whole repertoire of antigens challenges a node. In fact, the observed decrease in the percentage of precursor cells is explainable simply by a parallel increased production of lymphocytes by the precursor cells and the transient accumulation of their progeny lymphocytes in the deep cortex units where most lymphocytes occurred, as discussed by Bélisle and Sainte-Marie (1981).

Also supporting our conclusion, on selective colonization, is the meaningful later occurrence of a very high percentage of blast-related cells in the peripheries of occasional deep cortex units as earlier observed (see Figs. 12-13 in: Bélisle and Sainte-Marie, 1981). Related observations indicated that such cells in a unit periphery are on their way to contiguous medullary cords where they form plasmacytes, a conclusion supported by immunological studies (Roojen, 1987). The observation, therefore, appears to reflect a peculiar condition linked to humoral immunity which would favor an increased unfolding of blastogenesis of lymphocytes in a peripheral tissue rather than in the draining node. This would result in a greater arrival of blast-related cells via the concerned afferent lymphatic(s) and their migration into the periphery of the topographically related deep cortex unit(s), following the pattern of intranodal cell migration described by Sainte-Marie and Peng (1996). The explanation can account as well for the concomittant scarcity of lymphocytes in such a unit's periphery: an increased unfolding of blastogenesis of activated lymphocytes in the peripheral tissue would decrease their arrival in the periphery of concerned deep cortex unit(s). As to the limitation of the phenomenon to one or a few deep cortex units of a same node, it results from nodal compartmentation: events in a given compartment being usually influenced only with the content of the related afferent lymphatic vessel which can differ from that carried by other vessels (Sainte-Marie et al., 1975, 1982). Thus, the above occasional observation, made on days 7-13, lately betrays an antigen-specific entry of cells into nodes just as do findings made on day one.

In another respect, the early high relative abundance of blast-related cells in the subcapsular sinus of a

neonatal node, that reflects an antigen-specific entry of lymphoid cells in nodes, in turn may suggest a correspondingly specific entry of blood lymphocytes in the drained peripheral tissue. The alternative explanation would be that, among the naïve blood cells penetrating a peripheral tissue at random, virtually only those activated there enter the local lymph and node. The unactivated cells could be eliminated in situ after a while. A naïve cell, having spent some time in the blood circulation before entering a tissue where it further wanders for a while without being activated, might have consumed much to all of its brief lifespan since naïve cells live only a few days if unactivated (Stites et al., 1994). There would be little utility for such an aged cell to return to the blood traffic via lymphatics.

A last conclusion stems from the presence of lymphocytes in subcapsular sinuses on first days. The immunologic surveillance of peripheral tissues was attributed exclusively to memory lymphocytes. Such a cell would enter preferentially the tissue penetrated by the antigen having caused its formation whereas a naïve cell would enter randomly any node in quest of the antigen of its competence (Mackay, 1993; Adams and Shaw, 1994). But the presence of lymphocytes in the afferent lymph of nodes and in the lamina of intestinal villi, shortly after birth, indicates that, at least in neonates, naïve cells participate in peripheral surveillance. Otherwise, memory cells being initially inexistant, surveillance would not occur during this perilous neonatal period. Furthermore, and especially if entry of naïve cells in nodes occurred at random, such a situation would require a massive entry of these cells in each node almost immediately after birth (which has never been observed) so that rare competent cells could encounter drained antigens and rapidly develop immune responses providing memory-cells urgently needed for peripheral surveillance.

To summarize, the present data reveal that the earliest lymphoid colonization of neonatal rat nodes involves lymph-carried cells, but scarcely (if any) blood cells recruited by venules. Moreover, the initial predominance of nodal blast-related cells over lymphocytes is incompatible with the popular but unproven view of a random lymphoid cell recruitment by nodes. It indicates instead an antigen-specific process of colonization. Similarly, each of the previously examined many changes undergone by adult nodes under various immunological conditions revealed an antigen-specific entry of circulating lymphoid cells in nodes (Sainte-Marie and Peng, 1996). Hence, the present findings further support our previous conclusion that an initial event of a specific immune response, consisting in the direct or indirect recruitment of competent circulating lymphocytes by nodes, is antigen-specific as all other events of the response (Sainte-Marie and Peng, 1996). Lastly, the present findings provide a strong incentive, to attempt obtaining a direct experimental evidence of this conclusion in neonates.

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