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AGE AND SEX DIFFERENCES IN THE RESPONSE OF LYMPH NODE POST-CAPILLARY VENULES IN MICE INFECTED WITH *TOXOPLASMA GONDII*

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Summary.—Groups of male and female mice of varying ages were infected with a low-virulence strain of *Toxoplasma gondii* and killed 3 weeks and 6 weeks after infection. The lymph nodes of the female group showed a greater prominence of the endothelial cells of the post-capillary venules. This difference was most marked at the age of 15 weeks and least at 30 or more weeks. The same sex difference was demonstrated in control mice although to a lesser extent. The possible role is discussed of the effect of female sex hormones on vascular endothelium in modifying the development of an immune response and in explaining the observed female preponderance in human cases of auto-immune disease.

IN Britain, lymphadenopathy due to postnatally acquired toxoplasma infections in man is seen more frequently in males than females under the age of 15 yr, more frequently in women than men of 25 yr and over, while between these ages the sexes are affected equally (Beverley *et al.*, 1976). These authors have drawn attention to a similar age–sex distribution in several other European countries. There is no evidence that infection rates in the sexes differ according to age. The lymph nodes undergo a reactive hyperplasia which in some patients may be so exaggerated as to cause clinically manifest lymph node enlargement.

Strains of *Toxoplasma gondii* isolated from patients with toxoplasmic lymphadenopathy have, in our experience, all been of the low-virulence type. The histological changes caused by such a strain in rabbit lymph nodes (Henry *et al.*, 1973) and those caused in the nodes of male mice (Henry and Beverley, 1976) are similar to those found in man. An investigation of the response of the reticulo-endothelial (RE) systems of male and female mice at different ages has therefore been made and this paper is a report of the changes found in the endothelium of the post-capillary venules in lymph nodes.

MATERIALS AND METHODS

Mice were of a white non-inbred laboratory strain and were aged 4, 7, 10, 15, 20, 30 or 40 weeks at the time of inoculation.

The inoculum consisted of 20 toxoplasma tissue cysts harvested from mouse brains 9 weeks after infection and was given as a dilute brain emulsion s.c. The strain was first isolated from an apparently healthy rabbit and has since been maintained by s.c. passage at intervals of 3–4 months of brain cysts from the donor mice.

Twelve male mice and 12 females of each age group were used. Eight of the 12 were inoculated and 4 served as controls. Four of the inoculated and 2 controls were killed for examination 3 weeks after infection, the other 4 inoculated and 2 controls were killed 6 weeks after infection. These times for examination were chosen in the light of the findings already reported (Henry and Beverley, 1976).

Examinations.—Mice were exsanguinated under ether anaesthesia. Lymph nodes were obtained from the flanks or sometimes from the axilla when the former were not prominent. They were fixed in formalin and processed routinely to paraffin. Sections were cut at 5μ and stained by haematoxylin and eosin. Dye

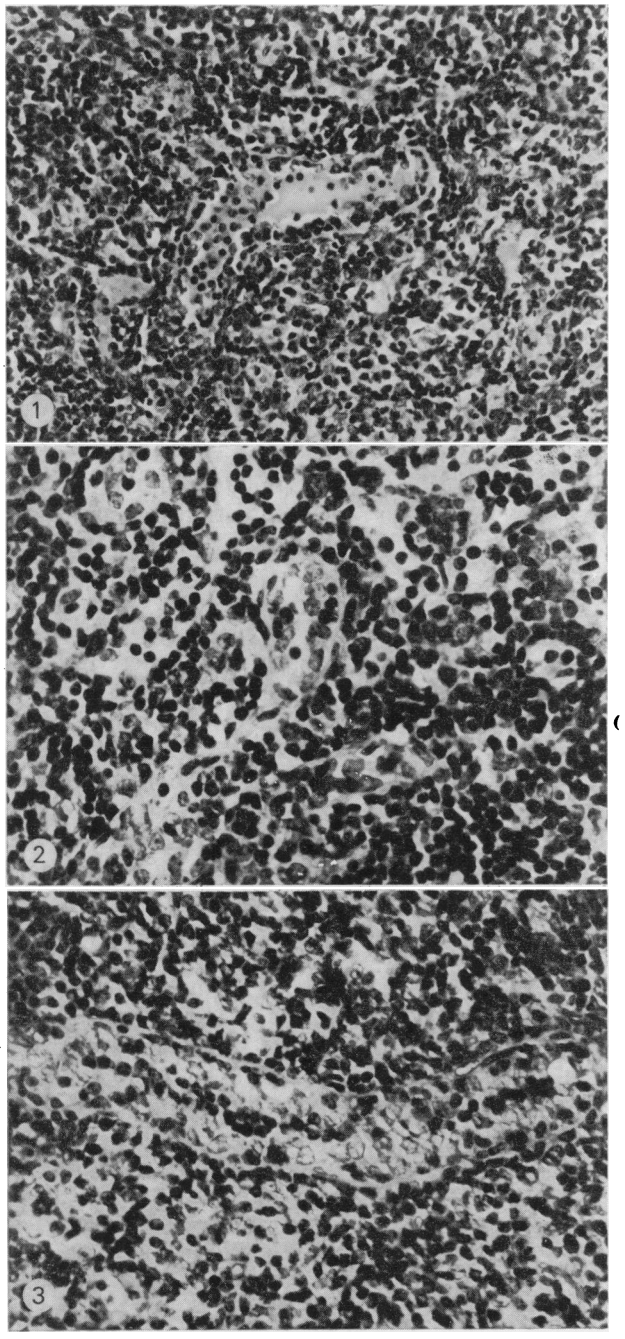

FIG. 1.—Female mouse 20 weeks. 6 weeks after inoculation. Lymph node post-capillary venule showing cuboidal epithelium. H. and E. ×320.

FIG. 2.—Female mouse 15 weeks. 6 weeks after inoculation. Lymph node post-capillary venule showing columnar epithelium. H. and E. ×320.

FIG. 3.—Female mouse 15 weeks. 6 weeks after inoculation. The lumen of the lymph node post-capillary venule is occluded by the swollen endothelial cells. H. and E. ×320.

tests for toxoplasma antibodies (Sabin and Feldman, 1948) were done on the sera obtained at necropsy. Further proof of toxoplasma infection was sought by grinding each mouse brain in 1·0 ml of isotonic saline and examining for the presence of toxoplasma tissue cysts in wet film preparations of the resulting emulsion.

In some of the control mice the lymph nodes were so small as to escape detection. One male and one female mouse in the 30-week group died before the 6 weeks after inoculation had elapsed.

Histological assessment

Post-capillary venules in the mouse lymph node are usually found in the paracortical area although they are occasionally present in the medulla. The resting endothelium is slightly more plump than that of capillaries or venules and as the node undergoes an immunological response the height of the endothelial cells becomes increased. The cells then become cuboidal (Fig. 1) and columnar (Fig. 2) with eventual obliteration of the lumen (Fig. 3). These appearances represent a spectrum of change but can be used to give a semi-quantitative assessment of the degree of reaction in the post-capillary venules as follows:

−	= resting endothelium
+	= endothelial cells more plump than in the resting state
++	= endothelial cells cuboidal
+++	= endothelial cells columnar
++++	= obliteration of the venular lumen

This grading is not only used to delineate the changes in individual venules but to give an assessment of the changes in the post-capillary venules of the node as a whole. In all but the resting state, lymphocytes are observed within the endothelial cell layer or between the endothelial cells and the venular basement membrane. This feature was not therefore used in assessing the degree of reaction.

Analysis of results

By converting the plus grading to a numerical 0–4 system a value can be assigned to the response of each group. Since the method of grading is necessarily imprecise no formal statistical analysis was undertaken. However, it is possible to calculate the standard error of the difference between groups. If the difference between the values in any two groups is greater than twice the standard error, it is regarded as significant. No attempt was made to assess the degree of significance in the form of a value for P.

RESULTS

In response to an antigenic stimulus the post-capillary venules of the lymph node undergo a spectrum of changes which, in the absence of an inflammatory component, should be regarded as physiological rather than pathological. Toxoplasmic lymphadenopathy represents such a non-inflammatory response (Henry *et al.*, 1973) and the results in Table I show the effect on the post-capillary venules in mice infected with a low-virulence strain of *T. gondii*. Infection was confirmed by a positive dye-test titre and the finding of cysts in the brain. The number of cysts at 6 weeks after infection compared with mice examined at 3 weeks show that the infection was passing from an active into a latent phase. On a semi-quantitative assessment a score of 28 was obtained in 49 uninfected control mice which differed significantly from a score of 92 in 110 infected mice, indicating a specific effect of the toxoplasma infection. The changes in the control mice presumably reflect a reaction to antigenic material in the environment. At 3 weeks after infection the score in the infected animals does not differ significantly from the controls but at 6 weeks a significant effect is seen, with a score of 57 in 54 infected mice as against 14 in 24 controls. The values in the control mice at 3 and 6 weeks respectively are almost identical.

Significant sex differences are apparent in both the control and infected mice. Twenty-five control males give a score of 5: 24 control females one of 23. Of the infected mice 55 males gave a score of 22 compared with 70 in the same number of females. The differences in the infected male and female mice are also significant when the 3-week and 6-week groups are considered separately. This difference is most apparent when the infection is in mice aged 15 weeks. The only age group where the male score exceeded that of the female was in the oldest mice, aged 40 weeks, in the infected group. This age distribution with a peak at 15 weeks of age is only seen in the infected female mice

TABLE I.—*Response of Lymph Node Post-capillary Venules to Infection with a Low Virulence Strain of* T. gondii *in Male and Female Mice of Varying Ages Compared with Uninfected Controls*

Age at infection (weeks)	Control mice				Infected mice			
	3 weeks		6 weeks		3 weeks		6 weeks	
	Male	Female	Male	Female	Male	Female	Male	Female
4	−	−	+	+	−	+	−	++
	+			+	−	+	+++	++
					−	+	−	++
					−	+	−	+
7	−	++	−	++	+	+	−	++
	+		−	+	+	−	+	++
					−	++	+	++
					−	+	−	++
10	−	++	−	−	−	+	+	++
	+			+	+	+	−	++
					−	++	−	+++
					−	+	−	−
15	+	+	−	++	−	+	++	++++
	−	+	−		−	+	+	++++
					−	−	−	+++
					−	−	−	+++
20	−	−	−	+	+	+	−	+
	−	−	−	++	−	+	−	++
					−	+	−	+++
					+	−	+	++
30	−	+	−	++	−	+	−	−
	−	+	−	−	−	+	−	−
					+	+	−	−
					−	+		
40	−	+	−	−	+	+	−	−
	−	+		−	+	+	−	+
					+	+	++	−
					+	−	−	−
Total	4	10	1	13	10	25	12	45
Mice	14	11	11	13	28	28	27	27

examined at 6 weeks. The remaining infected groups and the control mice did not show any age effect.

DISCUSSION

The observations of Beverley *et al.* (1976) indicate a sex difference in the response of lymph nodes in man to infection with *T. gondii*. The present study confirms that certain sex differences can be identified in mice infected with the low-virulence strain of this organism as well as in control mice of both sexes. Particular attention is drawn to the blood vessels of the lymph nodes and it is suggested that the observed sex difference may in part be reflected by the influence of oestrogens on the endothelium of the post-capillary venules in the paracortical areas of the node. It is thus significant that the older mice fail to show any sex difference in this respect and that the maximal response is seen in mature female mice.

Post-capillary venules are a specialized area of the blood vessel, lying between the capillary and the larger venule. The endothelium is somewhat higher than in the adjacent portions of the blood vessel. Changes in the post-capillary venule are seen in acute inflammation (Florey and Grant, 1961). They represent the site of action on the blood vessel of histamine and serotonin (Majno and Palade, 1961; Majno, Palade and Schoefl, 1961) and may be prominent in granuloma formation (Smith *et al.*, 1970). In the lymph node they form a part of the vascular pattern (Söderstrom, 1967), lying mainly in the paracortical areas. They are rarely

seen in the normal spleen or thymus but can be demonstrated in the tonsil and occasionally in Peyer's patches. There is a considerable circulation of lymphocytes through the normal lymph node. Hall and Morris (1965) estimate that only 4% of the lymphocytes leaving a node are actually formed therein. The site of passage of these from the blood stream into the node has been identified by Gowans and Knight (1964) as through the wall of the post-capillary venule. The manner of this passage is probably through the cytoplasm of the endothelial cell (Trowell, 1955; Marchesi and Gowans, 1964) although in states of active inflammation of the node with exudation of polymorph leucocytes, the latter pass between the endothelial cells at the intercellular junctions (Smith and Wood, 1949; Marchesi and Gowans, 1964). Since the lymphocytes in the paracortical area of the node are concerned in the development of delayed hypersensitivity, Gowans *et al.* (1962) have suggested that the site of interaction between the lymphocyte and antigen may be in relation to the post-capillary venule in this area. Alterations in lymph node post-capillary venules have also been demonstrated in a transplantation situation (Burwell, 1962). Delayed hypersensitivity occurs in response to infection with *T. gondii* (Frenkel, 1948) and it may be, therefore, that the changes observed in the endothelium of these venules during an immunological reaction do reflect the development of this type of immunity. The present study indicates that the reaction of the endothelial cells to infection with *T. gondii* is a late effect and thus concerned more with cellular than with humoral immunity.

There is no doubt that oestrogen does have an effect on vascular endothelium. After a single injection of oestradiol into female rats, Friederici (1967) found that up to 6 h later an increased permeability of the uterine capillaries could be demonstrated. Areas of separation were seen between the endothelial cells although the capillary basement membrane retained its integrity. After 20 h the uterus was hyperaemic but the capillary permeability had returned to normal. There was, however, a phase of myometrial growth and at this time the cytoplasm of the endothelial cells showed changes suggesting synthesis of molecular complexes. These findings were confirmed by Ham *et al.* (1970) who found that an injection of oestradiol induces a massive escape of fluid with the protein content of plasma into the extravascular tissue of the uterus of immature rats. Electronmicroscopy revealed transient and reversible gaps in the normally complete endothelial lining of capillaries and small venules, with later evidence of growth and secretory activity in the endothelial cells. Antihistamines had no effect in abolishing these changes but the morphology of the gaps in the endothelial cells was similar to that produced by histamine. This is not only seen with exogenous oestrogens but cyclical changes may also occur in blood vessels during oestrus. Burr and Davies (1951) found that the vascular permeability of capillaries and venules in the rabbit ovary was cyclically related to ovulation. Pappas and Blanchette (1965) showed that the previously mentioned findings in the rat uterus were reproduced by endogenous cyclic hormone secretion, the major effect being in the late proestrus–midoestrus period with little increase of permeability during dioestrus. The same authors also found the blood vessels of the Graafian follicle to be more permeable in the pre-ovulatory period with marker particles accumulating in the area of inter-endothelial cell junctions. The resulting pre-ovulatory swelling of the follicle with transudate of fluid may facilitate its rupture. In a similar fashion the flow of lymph from the ovary increases coincidentally with ovulation and the formation of a corpus luteum (Morris and Sass, 1966). The histological appearances of the endothelium of the lymph node post-capillary venules do not exactly correspond with those described in other organs in response to oestrogen stimulation. However, Hur-

ley and McCallum (1974) have shown that in rats and mice the post-capillary venules in Peyer's patches show an increased permeability, marker particles being found both within the cytoplasm of the endothelial cells and in relation to the intercellular junctions.

In the present study therefore it is postulated that in response to a specific antigenic stimulus, *i.e. T. gondii*, the hormonal milieu of the female may accentuate the changes in the endothelium of the lymph node post-capillary venules as compared with the male. This may produce histological differences in the node accounting for the higher proportion of females of child-bearing age showing toxoplasmic lymphadenopathy (Beverley *et al.*, 1976) and may also modify the immune response, although it is not asserted that the post-capillary venules play a sole or primary role in this respect; other factors may well be involved. The sex differences are quantitative rather than qualitative since the changes are seen in the post-capillary venules of males, but to a lesser extent. A sex difference is also present in the control mice, presumably because there is no occasion when the animal is not subject to some antigenic stimulus, unless kept under exceptional conditions. Söderstrom (1967) found that the endothelium of lymph node post-capillary venules was not prominent in young mice until 45 days of age, this development presumably resulting from normal exposure to antigenic stimuli in the environment. However, even under these conditions the present study indicates that uninfected female mice show a greater effect on the post-capillary venules than does the uninfected male.

These considerations may also have some bearing on the observed sex difference in cases of autoimmune disease in the human, where, in adults, there is a considerable female predominance (Beverley *et al.*, 1976). In such cases the thymus may show development of lymphoid follicles with germinal centres. There is normally a circulation of lymphocytes through the thymus (Sainte-Marie and Leblond, 1964) but the vascular endothelium is not normally of lymph-node type. However, in patients suffering from myasthenia gravis, not only are lymphoid follicles seen in the thymus but the endothelium of the associated post-capillary venules is also similar to that seen in lymph nodes (Söderstrom *et al.*, 1970). Bradfield (1973) showed similar findings. In 41 myasthenic patients, 19 showed thymic germinal centres and in 15 of these the endothelium of the post-capillary venules was of the high type. However, 5 of 15 control thymuses also showed germinal centres and in 3 of these the post-capillary venule endothelium was prominent. It may be that in females the effect of the sex hormones on the endothelial cells of post capillary venules in the RE system allows an abnormal contact between auto-antigens and immunologically competent cells resulting in the production of autoimmune disease.

Therefore, although the present study has been conducted with a specific antigenic stimulus, the effect of female sex hormones on vascular endothelium may well be of a general nature with wider implications involving the immune response and perhaps explaining in part the greater frequency of auto-immune disease in women than in men.

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