Changes in Splenic Microcirculatory Pathways in Chronic Idiopathic Thrombocytopenic Purpura

By E.E. Schmidt, I.C. MacDonald, and A.C. Groom

The spleen plays a central role in the pathogenesis of chronic idiopathic thrombocytopenic purpura (ITP); it produces massive quantities of antiplatelet antibodies, leading to accelerated phagocytosis of platelets. Lymphoid hyperplasia typically occurs in the spleen, characterized by large numbers of lymphatic nodules with active germinal centers. Whether changes in splenic microcirculatory pathways also occur is not known. We have studied this question by scanning electron microscopy of corrosion casts, comparing spleens removed from patients with ITP with normal spleens obtained from organ transplant donors. The casts demonstrate two major changes in microcirculatory pathways in ITP. Firstly, a striking proliferation of arterioles and capillaries is found in the white pulp and marginal zone (MZ), seen as extensive vascularization in 92.3% of lymphatic nodules (n = 191) versus 0.6% (n = 224) in normal spleens. Secondly, the marginal sinus, a series of flattened, anastomosing vascular spaces between the white pulp and MZ, is absent in 89.4% of lymphatic nodules versus 4.9% in normal spleens. The cause of these microcirculatory changes, which may not be exclusive to ITP, is presently unknown. Absence of the marginal sinus may affect distribution of blood flow through the MZ such that platelets spend increased amounts of time in the proximity of macrophages. In the presence of antiplatelet antibodies found in ITP spleens, this delayed transit would lead to greatly increased platelet destruction.

RESULTS

The clinical data for the seven patients with ITP, aged 20 to 76 years, are summarized in Table 1. The time elapsed from diagnosis of the disease until splenectomy ranged from 6 weeks to 2.25 years. During this interval all patients received corticosteroid therapy (prednisone). In some cases, immunosuppressive therapy (vincristine) was also administered initially. Approximately 1 day before splenectomy, all patients received a polyvalent pneumococcal vaccine (pneumovax, 0.5 mL). Splenic weights ranged from 120 to 362 g (mean, 214 ± 2 g [SD]).

The microcorrosion casts demonstrated two major differences in microcirculatory pathways of ITP spleens as compared with normals. First, a great proliferation of arterioles and capillaries was found in the white pulp and marginal zone (MZ).
marginal zone (MZ) of ITP spleens. The three-dimensional relationship between the arterial tree, a lymphatic nodule, and the surrounding marginal sinus (MS) and MZ is seen in Fig 1a, taken from a cast of normal spleen. Two arterioles from within the nodule pass out into the MZ, where they give rise to capillaries that curve circumferentially before terminating in the MZ. Follicular capillaries are absent in this cast, a common finding in normal spleens (as explained below in Table 2). In contrast to the situation in normal spleens, a striking increase in the degree of arterial branching within the white pulp and MZ was found in ITP spleens. In the example shown (Fig 1b), numerous bundles of small arterioles and capillaries are seen within the nodule. Most of these vessels pass out into the MZ that borders the nodule.

Casts of 191 lymphatic nodules from seven spleens of patients with ITP were examined for the presence of small arterioles and capillaries within the white pulp and surrounding MZ. For each nodule the degree of vascularization was ranked as ‘extensive,’ ‘moderate,’ or ‘sparse.’ The results were compared with corresponding data from casts of 224 nodules from eight normal spleens (Table 2). Percentage values are given for each individual spleen. These values indicate that in three of the ITP spleens 100% of the nodules examined showed extensive vascularization. The lowest value encountered was 78%. In contrast, in normal spleens the highest percentage of nodules showing extensive vascularization was 3%, with six of the eight spleens yielding a value of 0%. Overall, vascularization was extensive in 92.3% of nodules from ITP spleens, moderate in 6.4%, and sparse in 1.3%. In contrast, for normal spleens the corresponding numbers were 0.6%, 16.3%, and 83.1%, respectively. These data show that there is a consistent increase in vascularity in and around lymphatic nodules in ITP spleens.

The second difference in microcirculatory pathways of ITP spleens, as compared with normals, was the absence of an MS in ITP spleens. The casts show that the MS is a very distinct entity in normal spleens, consisting of a series of flattened, anastomosing vascular spaces lying between the white pulp and MZ (Fig 2a). The sheet-like appearance of MS casts is quite different from the knobbly configuration of casts of the adjacent MZ meshwork. At the upper right of Fig 2a (→), the MS cast appears extremely thin and fragmented, due to incomplete filling; in other areas in which filling is complete, the thickness of the MS is still only 10 μm or less. An opening in the MS is visible (Fig 2a, *), representing the site where the central artery (cast accidentally broken off during processing) entered the nodule. Numerous apertures in the wall of the MS on the side facing the MZ (not shown) allow material to flow outwards into the MZ and on into the red pulp. In contrast to casts from normal spleens, in which MS filling could be observed around most lymphatic nodules, casts from ITP spleens consistently lacked an MS (except for small isolated patches found infrequently). In the example shown (Fig 2b), the cast bordering the region formerly occupied by a lymphatic nodule has the knobbly appearance characteristic of MZ

<table>
<thead>
<tr>
<th>Spleen No.</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Duration of Disease</th>
<th>Splenic Weight (g)</th>
<th>Prednisone Dose (mg/d)</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53</td>
<td>F</td>
<td>9 mo</td>
<td>121</td>
<td>30 → 10</td>
<td>7 mo</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>F</td>
<td>7 mo</td>
<td>150</td>
<td>40 → 15</td>
<td>7 mo</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>F</td>
<td>2.25 y</td>
<td>212</td>
<td>80 → 5</td>
<td>2.25 y</td>
</tr>
<tr>
<td>4</td>
<td>76</td>
<td>F</td>
<td>2 y</td>
<td>120</td>
<td>50 → 5*</td>
<td>2 y</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>M</td>
<td>6 mo</td>
<td>300</td>
<td>50 → 20*</td>
<td>6 mo</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>M</td>
<td>6 wk</td>
<td>235</td>
<td>75*</td>
<td>6 wk</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>F</td>
<td>3 mo</td>
<td>235</td>
<td>50*</td>
<td>3 mo</td>
</tr>
</tbody>
</table>

*Vincristine was also administered initially in three doses of 2 mg weekly.
Table 2. Percentage of Lymphatic Nodules, in ITP Versus Normal Spleens, Showing Different Grades of Vascularization and Presence of an MS

<table>
<thead>
<tr>
<th>Spleen No.</th>
<th>Vascularization</th>
<th>MS</th>
<th>No. of Nodules Examine</th>
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<tbody>
<tr>
<td>ITP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>84</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>92</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>92</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>78</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>92.3</td>
<td>6.4</td>
<td>1.3</td>
</tr>
<tr>
<td>± SD</td>
<td>8.7</td>
<td>6.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Normal†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>45</td>
<td>52</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>24</td>
<td>74</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>7</td>
<td>93</td>
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<td>14</td>
<td>0</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td>Mean</td>
<td>0.6</td>
<td>16.3</td>
<td>83.1</td>
</tr>
<tr>
<td>± SD</td>
<td>1.2</td>
<td>13.7</td>
<td>14.8</td>
</tr>
</tbody>
</table>

*Percentage indeterminate. The amount of casting compound reaching the blood space bordering the white pulp was insufficient for the presence of an MS to be determined.
†Normal spleens were from transplant donors, age (y) and sex as follows: 8 (42,F), 9 (38,F), 10 (2,M), 11 (10,F), 12 (11,M), 13 (62,F), 14 (56,F), 15 (33,M).

filling. Several capillaries (*) run circumferentially in the MZ and terminate there, but no evidence of an MS is present. Similarly, the cast in Fig 1b also lacks an MS. In each of the casts mentioned above, lymphatic nodules were examined not only for the degree of vascularization but also for the presence or absence of an MS. For each nodule the MS was ranked as ‘extensive,’ ‘incomplete,’ or ‘absent.’ The results (Table 2) show that in ITP spleens not even one of the 191 nodules examined had an extensive MS; in 10.6% of the nodules the MS was incomplete; and in 89.4% the MS was absent. In contrast, in normal spleens an average of 31.5% of nodules had an extensive MS, 63.6% had an incomplete MS, and in only 4.9% was the MS absent. These data show that there is a consistent loss of MS around lymphatic nodules in ITP spleens.

Table 3 shows the pronounced association in ITP spleens
vascularization and absence of the MS (92.3%/89.4%) was
were so dramatic that casts from each group were immedi-
with tremendous reduction in the number of vessels in each
lymphatic nodules and the surrounding MZ, and (2)
very pronounced in ITP spleens, as was the association
absence of the MS bordering the white pulp. These changes
absence of the MS. Table 3 also shows a strong association
in normal spleens between sparse to moderate vascularization
in ITP spleens versus only 0.6% in normal spleens. The MS
absence of the MS in human spleen is not a species characteristic, as
suggested by Van Krieken et al., but a consequence of a
disease state such as ITP.

Corrosion casts have demonstrated in other mammalian
spleens that the MS is a distinct vascular space that has a
plentiful blood supply, with some arterioles capillaries termini-
ating on its inner and others on its outer aspect. The MS
fills preferentially, before filling of the MS and surrounding
red pulp occurs. Flow through the MS distributes blood
uniformly to the MZ around each lymphatic nodule. In our
casts from normal human spleen, preferential filling of the MS (ie, before the MZ) is not as clear as in some other
species, but significant consequences may result when the
MS is absent, such as in ITP. For example, blood flow
through the MZ could become less uniformly distributed,
giving rise to areas of slow flow; this would cause platelets
(and other blood cells) to spend increased time in the
proximity of splenic macrophages. This change, along with
the presence of high concentrations of antiplatelet anti-
body, could lead to the accelerated destruction of plate-
lets characteristic of ITP.

The present results show, unequivocally, that hypervascu-
larization and the absence of the MS bordering splenic
lymphatic nodules are changes that occur in ITP. However,
it should not be concluded that these changes are specific to
ITP alone. The changes could be a reflection of follicular
hyperplasia, which may occur in several disorders. We were
not able to obtain spleens from patients with other immune
disorders, but in splenic casts from two patients with
hypersplenism and one with chronic lymphocytic leukemia
we found similar changes. (Extensive vascularization was
present in 71% to 100% of 59 nodules examined and the
MS was absent in 69% to 82%.) This result suggests that the
above changes are not specific to ITP alone.

Because all the ITP patients had been treated with
prednisone before splenectomy (for periods of 6 weeks to
2.25 years), the question arises as to the possibility that the
changes observed in this study could have been the result of
steroid therapy? We cannot rule out this possibility, but we
regard it as improbable for the following reasons: (1)
Although no spleens were available to us from ITP patients
who had not received steroids, similar changes can occur in
the absence of steroid therapy, as shown by our casts from the
hypersplenism patients mentioned above. (2) In the

Table 3. Inverse Relationship Between Vascularization of Lymphatic
Nodules and the Presence of an MS in ITP Versus Normal Spleens

<table>
<thead>
<tr>
<th>Vascularization</th>
<th>Vascularization Sparse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensive/</td>
<td>MS Absent</td>
</tr>
<tr>
<td>92.3</td>
<td>7.7</td>
</tr>
<tr>
<td>89.4</td>
<td>10.6</td>
</tr>
<tr>
<td>0.6</td>
<td>99.4</td>
</tr>
<tr>
<td>4.9</td>
<td>95.1</td>
</tr>
</tbody>
</table>

Numbers indicate percentage of nodules in each category (see Table 2).

between extensive vascularization of the nodule and the
absence of an MS. Table 3 also shows a strong association
in normal spleens between sparse to moderate vascularization
and the presence of an MS. Furthermore, it is evident that
in ITP the presence of even an incomplete MS is associated
with tremendous reduction in the number of vessels in each
nodule and its surrounding MZ.

DISCUSSION

Two changes in splenic microcirculatory pathways were
found in patients with chronic ITP, as compared to normal
subjects: (1) proliferation of arterioles and capillaries
within lymphatic nodules and the surrounding MZ, and (2)
absence of the MS bordering the white pulp. These changes
were so dramatic that casts from each group were immedi-
ately identifiable on viewing under SEM, precluding the use
of “blind” measurements. To evaluate the consistency of
these findings, we examined casts of a large number of
lymphatic nodules (n = 191, ITP; n = 224, normal) and
graded both the degree of vascularization and the extent of
the MS into three categories. When the results for each
category were expressed as a percentage of the total
number of nodules examined for each spleen, the consis-
tency of the changes in ITP became very clear. In addition,
these numerical data showed the magnitude of the changes.
Extensive vascularization was present in 92.3% of nodules
in ITP spleens versus only 0.6% in normal spleens. The MS
was absent in 89.4% of nodules in ITP spleens versus 4.9%
in normal spleens. Thus, absence of the MS is associated
with high concentrations of antiplatelet antibo,
and the presence of an MS. Furthermore, it is evident that

The presence of the MS is difficult to demonstrate by
means of histologic sections, although at the boundary of
the white pulp a structure referred to as “layers of circum-
ferential reticulum” has been reported.11,12 Indeed, in a
recent report it was concluded that the human spleen lacks
an MS.13 The first clear evidence for the existence and
three-dimensional morphology of the MS in normal human
spleen has been provided by SEM of microcorrosion casts.7
The present study adds quantitative data regarding the
incidence of the MS, inasmuch as this structure was found
to be present in 95.1% of the 224 lymphatic nodules
examined from eight normal spleens. In marked contrast to
this, the MS was absent in 89.4% of the 191 nodules
examined in the seven ITP spleens. Thus, absence of the
MS in human spleen is not a species characteristic, as
suggested by Van Krieken et al, but a consequence of a
disease state such as ITP.

To our knowledge, the only previous report of vessel
proliferation in ITP spleens is that of Tavassoli and Mc-
Millan.1 From transmission electron micrographs they ob-
served an unusually large number of vessels in the MZ
surrounding lymphatic nodules. Quantitative evidence for
this finding was not provided, nor did they report on
vascularization within the nodules. From a morphometric
study based on light microscopy of histologic sections, Van
Krieken et al10 concluded that there was no increase in
vascularity in the white pulp of ITP spleens. However, at
the low magnifications used in their study it seems likely
that some of the small vessels would have been missed,
obscuring changes in vascularization that may have been
present.
study by Hayes et al., some ITP patients had received steroids, while others had received no therapy before splenectomy. The investigators found no differences in splenic histopathology between the groups.

What could be the cause of this microvascular proliferation within and around lymphatic nodules in ITP spleens? Jäger’s observations on normal spleens may have some bearing on this matter. He found a variation in the degree of vascularity within nodules, and suggested that this is related to different phases of activity through which a given nodule may pass. In large “blossoming” nodules with active germinal centers he found a rich “inner network” of follicular capillaries and an “outer network” of arterioles and capillaries in the MZ. However, in smaller nodules lacking a germinal center (which had regressed into an “involution” phase) the inner network of vessels was greatly reduced or absent, and only the outer network remained. Our casts of normal spleens showed that, on average, vascularization was extensive in less than 1% of nodules, moderate in 16%, and sparse in 83%. These results could reflect different phases of nodular activity, as proposed by Jäger. In relation to ITP spleens, Jäger’s findings suggest that the proliferation of vessels within nodules may have arisen in association with the development of highly active germinal centers characteristic of this disease. Further support for this conclusion comes from the finding that there is an increase in capillary density in peripheral lymph nodes undergoing immunologic reactions. Macrophages are an important component of such immunologic reactions, and in ITP macrophages are especially plentiful and active in phagocytosis of platelets within splenic nodules (as well as in the MZ and red pulp). The finding that activated macrophages induce vascular proliferation suggests the possibility that a macrophage-derived growth factor may mediate microvascular proliferation within and around splenic lymphatic nodules in ITP.

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