Immunofluorescence of Megakaryocytes in the Thrombocytopenic Purpuras

By F. PIZZI, P. M. CARRARA, A. ALDECHI AND S. ERIDANI

THE METHOD of Coons and Kaplan for the detection of antigens in tissue cells using fluorescent antibodies has been successfully applied to the study of platelet immunology. Humphrey8, Silber et al.12 and Vasquez and Lewis13 employed fluorescein-labeled antiplatelet serum to establish immunologic identity between platelets and the cytoplasm of megakaryocytes. Craig and Gitlin2 used fluorescent techniques to determine the nature of the material in the hyaline thrombi in thrombotic thrombocytopenic purpura. McKenna and Pisciotta8 used fluorescent antiglobulin serum to demonstrate protein adhesion to the cytoplasm of megakaryocytes in some cases of chronic ITP. With the same purpose Ebbe, Wittels and Dameshek3 used antihuman globulin conjugated with fluorescein isothiocyanate in two cases of autoimmune thrombocytopenic purpura (ITP type) associated with chronic lymphocytic leukemia.

Using the same method, the present investigation was used to confirm the possibility of an immunologic component in some cases of Werlhof’s syndrome and to develop additional information on the type of the protein involved.

The study was performed in 10 normal subjects and in 34 cases of thrombocytopenic purpura, 16 of which were examples of the chronic and 2 of the acute variety of ITP, 4 of thrombocytopenias with bone marrow aplasia, 2 in the course of multiple myeloma, 2 in the course of Moschowitz disease, 4 associated with acute leukemia, and 4 associated with SLE.

METHOD

Bone marrow tissue obtained by sternal biopsy was washed 6 times in saline buffered at pH 7, then squashed on glass slides and fixed 10 minutes in 95° ethanol. After a further washing in tap water for 15 minutes, the slides were dried in air, the fluorescent serum was applied and allowed to stand 1 hour at room temperature. The slide was then washed in tap water for 2 hours and, after air drying, was ready for examination.

The fluorescent serum was previously treated with a pool of human platelets, washed twice, obtained from subjects with different blood groups (A, B, O). Desiccated bone marrow was then added to the fluorescent serum to reduce the aspecific fluorescence.

An anti-β2 (IgA) globulin fluorescent serum was found the most suitable among the different ones tested (antihuman globulin serum, antigamma globulin serum, anti-β2 globulin serum).

In each case the following tests were performed: the direct test (megakaryocytes + anti-β2 globulin fluorescent serum); the indirect test (normal human megakaryocytes, incubated

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First submitted April 27, 1965; accepted for publication Oct. 25, 1965.

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Table 1

<table>
<thead>
<tr>
<th>Cases</th>
<th>N.</th>
<th>Positive Test</th>
<th>Negative Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Chronic ITP</td>
<td>16</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Acute ITP</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Myeloma</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Medullary aplasia</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Moschowitz syndrome</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>SLE</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>10</td>
<td>34</td>
</tr>
</tbody>
</table>

with serum of thrombocytopenic patients + anti-$\beta_2$ globulin fluorescent serum); and the blocking test (megakaryocytes incubated with nonfluorescent anti-$\beta_2$ globulin serum + fluorescent anti-$\beta_2$ globulin serum).

The evaluation of the test was performed according to the following criteria:
1. Diffusion and uniform fluorescence of the megakaryocyte's cytoplasm by the direct test
2. Normal granular fluorescence of the same area by the indirect test
3. Considerable decrease of fluorescence by the blocking test.

The test was considered positive for each case when all three reactions behaved as illustrated.

RESULTS

The results are presented in Table 1.

The morphologic features presented in Figures 1 and 2 show a bright and uniform cell fluorescence; the cytoplasm of megakaryocytes looks platelet-free, and the nucleus is dark without any fluorescence. These figures refer to cases of chronic ITP. In fact, only in some of these cases was the test positive. The positivity was confirmed by the blocking test (fig. 3).

Anti-$\gamma\alpha$ serum was found to induce an unequivocal fluorescence of megakaryocytes in all positive cases, while other sera, such as anti-human globulin and Anti-$\gamma\gamma$ gave inconstant and contradictory results. No evidence of cell fluorescence was observed in two cases of acute ITP.

In other thrombocytopenic syndromes, as in the course of multiple myeloma, Moschowitz disease and SLE, pale fluorescence of megakaryocyte cytoplasm, were occasionally observed (fig. 4), but the test was not considered positive because of a negative indirect test and because of the persistence of the finding after the blocking test.

Nuclear fluorescence was never observed except in one case of apparently idiopathic thrombocytopenic purpura, which finally gave place, after splenectomy, to a picture of SLE as reported elsewhere.10

A final observation concerns the frequent break of the cell membrane in cases of chronic ITP, as illustrated in Figures 2 and 3.

DISCUSSION

The results of the present investigation suggest that the use of the fluorescent antibody test according to Coons may be valuable for the assessment of the immunologic properties of megakaryocytes in the thrombocytopenic purpuras, with particular regard to the chronic variety of ITP.5,9,11
Fig. 1.—Strong cytoplasmatic fluorescence in two megakaryocytes in chronic ITP.

Fig. 2.—Strong cytoplasmatic fluorescence in two megakaryocytes in chronic ITP. Note the loss of continuity of the cellular membrane.
Fig. 3.—Blocking test in a megakaryocyte in chronic ITP with positivity of the direct and indirect test.

Fig. 4.—Pale cytoplasmatic fluorescence in two megakaryocytes in SLE.
In this form more than 50 per cent of the reported cases showed the presence of a protein material adherent to the surface of megakaryocytes, not removed by repeated washings, transferable to normal magakaryocytes, and almost completely blocked by a previous treatment with nonfluorescent specific antiserum.

The use of this test suggests the hypothesis that the presence of $\beta_2$A (IgA) globulin material coating the cell surface of megakaryocytes may be a distinctive feature of some cases of chronic ITP with megakaryocytic hyperplasia of the bone marrow. This finding was not observed in thrombocytopenias secondary to such conditions as hypoplastic anemia and leukemia.

**SUMMARY**

The fluorescent antibody technic was applied to the study of thrombocytopenic purpuras with a presumable immunologic pathogenesis.

The results of the investigation suggest the hypothesis that some plasma protein material is strongly adherent to the surface of megakaryocytes in more than 50 per cent of cases of chronic ITP.

**ACKNOWLEDGMENT**

We wish to thank Prof. Pernis of the Clinica del Lavoro of the University of Milan for kindly supplying the fluorescent serums.

**REFERENCES**

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