The Cytologic Reaction of the Spleen in Experimental Amyloidosis in Mice

By Avinoam Zlotnick and Chloe Tal

Amyloidosis has been experimentally produced in mice by repeated injections of sodium caseinate, of Freund's adjuvant, and of Freund's adjuvant plus brain. Teilum produced the condition in rabbits by hyperimmunization with Pfeiffer's bacillus. In the spleens of the animals he found a marked proliferation of plasma cells, many of which stained positively with periodic acid-Schiff (PAS).

Since there has been little detailed comment on the cytology of the spleen in animal amyloidosis, it was thought that further study of this aspect might help clarify the intricate mechanism underlying the production of amyloid. With this aim in view, the reactions of mice to the injection of both weak and strong antigens were investigated.

Materials and Methods

The experiments were conducted on 90 mice, W-Swiss H line, inbred, 6 weeks old, of both sexes, weighing 20 to 25 Gm. The animals were divided into one control group of 20 mice and three experimental groups. The experimental groups were injected as follows: One group received adjuvant alone, the second group, adjuvant plus TAB vaccine prepared according to Felix et al., and the third group, TAB vaccine alone.

The method of preparation of the adjuvant has been previously reported. The adjuvant plus TAB vaccine was prepared as follows: 31 cc. of TAB vaccine (dry weight 8 mg/cc.), 19 cc. saline, 50 cc. Bayol F, 250 mg. mycobacterium tuberculosis type H37RV var. hominis, and 20 mg. merthiolate were mixed in a Waring Blender for 4 minutes. All the mixtures were kept at 4 C. throughout the experiments. Each animal in groups 1 and 2 received 6 subcutaneous injections of 0.3 cc. each, at weekly intervals. The animals in the third group received 0.5 cc. of the TAB vaccine intraperitoneally thrice weekly.

Three spleen biopsies were performed on each animal from the experimental groups. These were taken a week prior to immunization and before the second and third injections. A fourth specimen was taken immediately after the animals were killed by decapitation, seven weeks after the first injection. Smears were immediately made, fixed for 3 minutes with methanol and stained with the May-Grunwald-Giemsa combination. Two smears from each animal were first examined. Those displaying a vivid proliferation of plasma cells were also stained with PAS and Methyl-violet. Spleen specimen were also immediately fixed in a 10 per cent formaldehyde solution. Paraffin embedded sections were prepared and stained with hematoxylin and eosin, and Congo red for amyloid. When the animals were killed, the blood of each group was pooled and the serum collected for paper electrophoresis. No biopsies were performed on the control animals.

From the Department of Internal Medicine A and the Department of Nervous Diseases, Laboratory of Experimental Neurology, The Rothschild-Hadassah University Hospital and the Hebrew University-Hadassah Medical School, Jerusalem, Israel.

This study was supported partly by the Julius and Marie Schneider Neuropsychiatric Fund and partly by the Hebrew University-Hadassah Medical School Research Fund.

We wish to thank Prof. J. J. Groen for his valuable suggestions and criticism and Mr. N. Conforti for his unstinting assistance.

Submitted Feb. 16, 1960; accepted for publication Mar. 24, 1960.
but they were killed at the end of the experiment and their blood was pooled for paper electrophoresis.

Results

The Cytology of the Spleen in Normal Controls

The differentiation of the various cell types found in smears of the spleen of normal mice presents some difficulty. As in the bone marrow of these animals, this is due to the paucity or practical absence of granules in the cytoplasm of the granulocytic series, as well as their special mode of maturation. The following cell types of the granulocytic series can be distinguished in smears of the normal spleen.

The myeloblast.—The myeloblast is a large cell characterized by a narrow band of blue, granule-free cytoplasm and a large nucleus containing several nucleoli. It is usually found in groups together with other young forms of the granulocytes. (fig. 5).

The intermediate cell.—The intermediate cell is the next stage in the process of maturation of the granulocyte. It is similar to the myeloblast but has lost the nucleoli. Its cytoplasm may contain some azurophil granules. This cell is probably equivalent to the promyelocyte of the human bone marrow.

The ring cell.—In the center of the nucleus of the intermediate cell a small aperture appears which gradually becomes larger until the nucleus takes on the appearance of a ring (fig. 5). At one point the ring thins and breaks. The cell then appears as a band form, from which the mature neutrophil develops.

In addition, spleen smears of uninjected mice display a homogeneous distribution of large and small lymphocytes, among which are scattered erythroblasts in different stages of maturity (fig. 1). A characteristic feature of these smears is the presence of a varying number of giant cells, exactly resembling megakaryocytes but showing no contained or surrounding thrombocytes (fig. 2). In thick parts of the smears mast cells are also found (fig. 1). Plasma cells are very rarely found in smears from spleens of nonimmunized animals.

Reaction to Immunization

The results in the 61 injected animals which survived are presented in table 1. The three groups of immunized mice responded similarly to the different antigens. The cytologic response of the spleen was characterized either by a marked proliferation of plasma cells and plasmacytoid reticulum cells (fig. 3) or by increased granulopoiesis (fig. 4), or by both reactions occurring simultaneously. Histologically amyloid deposits in varying quantities (fig. 12), were observed in 48 of 61 immunized animals.

Increased granulopoiesis occurred in only 37 of the immunized animals. It manifested itself by the appearance of many myeloblasts, intermediate cells, ring cells (fig. 4), and a considerable number of cells in various stages of mitosis (fig. 6).

Proliferation of plasma cells is the most characteristic reaction of the spleen to an antigenic stimulus. It was found in 46 of the 61 immunized ani-
Fig. 1 (top left).—Spleen smear of a nonimmunized mouse. Normoblasts are scattered between the lymphocytes. At the top of the picture a tissue mast cell is seen. Note the homogeneous picture of the smear. (× 460, reduced)

Fig. 2 (top right).—Same smear. Giant cell. (× 460, reduced)

Fig. 3 (center, left).—Reaction to immunization. Appearance of plasma cells. Normoblasts are scanty. (× 1000, reduced)

Fig. 4 (center, right).—Reaction to immunization. Increased granulopoiesis. (× 460, reduced)

Fig. 5 (bottom, left).—Two myeloblasts with several nucleoli and ring cells in different stages of development. (× 1500, reduced)

Fig. 6 (bottom, right).—Cell in mitosis. (× 1500, reduced)

The number of plasma cells varied from animal to animal and even in different parts of the same smear. Plasma cells with 2 or 3 nuclei were a common finding (fig. 8) and in some of these cells budding of the nucleus was observed. There was also an increased number of plasmacytoid reticulum cells. "Grape cells" were rarely found. In amyloid-bearing animals, in some plasma cells the cytoplasm had lost its structure and had become homo-
Table 1.—Results of Immunization with 2 Different Antigens

<table>
<thead>
<tr>
<th>Antigens</th>
<th>No. of animals</th>
<th>Increased granulopoiesis</th>
<th>Plasma cell hyperplasia</th>
<th>Flame cell</th>
<th>Amyloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjuvant</td>
<td>38</td>
<td>21</td>
<td>27</td>
<td>18</td>
<td>32</td>
</tr>
<tr>
<td>TAB + adjuvant</td>
<td>15</td>
<td>12</td>
<td>11</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>TAB</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>37</td>
<td>46</td>
<td>28</td>
<td>48</td>
</tr>
</tbody>
</table>

geneous, staining less intensely blue with M.G.G. (figs. 7 and 8). The cytoplasm at the periphery of some cells stained patchily with a reddish tinge. In other cells the whole of the cytoplasm was thus stained (fig. 9). Many plasma-cytoid reticulum cells stained in the same manner, their cytoplasm appearing as coagulated homogeneous reddish clumps (figs. 9 and 10). When this occurred, the cells disintegrated, and the contained substance was liberated into the intercellular space (fig. 11). These reddish cells have been called “flame cells.” In our studies they stained metachromatically with methyl violet, and were faintly positive with PAS. “Grape cells,” which stain strongly positive with PAS, are not stained by methyl violet. These “flame cells” were found in 28 of 48 animals with amyloid deposits. Their number varied in different animals and indifferent parts of the same smear.

Electrophoretic Studies

Table 2 presents the results of electrophoretic studies on the pooled sera of the controls and of the experimental groups of mice immunized with adjuvant alone, with TAB alone and with TAB plus adjuvant. In all experimental groups the level of albumin was decreased and that of the globulins was increased. In animals immunized with adjuvant alone there was a marked increase in the beta globulins, the increase in the gamma globulins being very slight, while in animals immunized with TAB vaccine alone or with TAB plus adjuvant there was a marked increase in the gamma globulins, with little increase in the beta globulins.

DISCUSSION

The origin of amyloid is subject to debate and provides a fertile field for hypotheses. Experimental amyloidosis, first produced by injection of sodium caseinate, has since been induced by many other substances such as turpentine, sulfur, human sera, various bacteria and multiple vaccines. It was found that the rate of amyloid production is determined by the number of injections rather than by the quantity of material injected. This led Letterer to suggest that amyloid might be a precipitated antibody. This hypothesis became of special interest when it was shown that hyperglobulinemia might be an important factor in the etiology of amyloidosis and when a close relationship was demonstrated between hyperglobulinemia, plasma cell hyperplasia and antibody formation. Teilum, drawing attention to the coincidence of hyperglobulineima, paramyloidosis and increase of plasma cells, attributed to the plasma cell the capacity to produce amyloid. Bayrd and Bennett detected amyloid in the plasma cells in a case of multiple myeloma,
but direct proof that amyloid is produced by plasma cells was lacking. Teilum later showed that in hyperimmune mice and rabbits there was a marked proliferation of pyroninophil reticuloendothelial cells, many of which lost their pyroninophilia and stained positively with PAS. He concluded that these
Table 2.—Paper Electrophoresis of Serum Proteins Before and After Immunization

<table>
<thead>
<tr>
<th></th>
<th>Total proteins (Gm. %)</th>
<th>Albumin + α-globulin (Gm. %)</th>
<th>α2-globulin (Gm. %)</th>
<th>β-globulin (Gm. %)</th>
<th>γ-globulin (Gm. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>5.7 49.4 2.8</td>
<td>17.6 1.0</td>
<td>19.0 1.1</td>
<td>14.0 0.8</td>
<td></td>
</tr>
<tr>
<td>Adjuvant</td>
<td>5.7 26.2 1.5</td>
<td>22.1 1.2</td>
<td>34.5 2.0</td>
<td>17.2 1.0</td>
<td></td>
</tr>
<tr>
<td>TAB + adjuvant</td>
<td>6.7 35.9 2.4</td>
<td>22.3 1.5</td>
<td>18.8 1.3</td>
<td>23.0 1.5</td>
<td></td>
</tr>
<tr>
<td>TAB</td>
<td>5.9 28.6 1.7</td>
<td>18.2 1.1</td>
<td>21.5 1.3</td>
<td>31.6 1.8</td>
<td></td>
</tr>
</tbody>
</table>

cells were producing amyloid. He also observed that cortisone or ACTH administration caused the disappearance of plasma cells in hyperimmune animals and enhanced amyloid production.4

The aim of the present experiments was to find out whether weak and strong antigens could produce different types of reactions. The cytologic reactions of the mice to the two antigens were similar. There was a proliferation of plasma cells and of plasmacytoid reticulum cells, which became partly transformed into “flame cells.” In the course of this process the cells seemed to produce amyloid. The electrophoretic patterns of the serum differed, there being a rise in beta globulins after injection of tubercle bacilli and in gamma globulins after injection of typhoid bacilli. It thus seems that the production of amyloid does not depend on the type of antigen used and that it may be associated with marked hyperproduction of either beta or gamma globulins. It is of interest that Vasquez and Dixon20 found gamma globulin in amyloid experimentally produced in rabbits by injection of caseinate or RNA. There was also an associated elevation of serum gamma globulins.

Teilum stressed the importance of the giant cells in the production of amyloid, since they stained positively with PAS.4 However, our observations showed these cells to be present before immunization, and thus their role in amyloid production cannot be as important as assumed. This author also considered the disappearance of the pyroninophil cells and their transformation into PAS-positive cells to be evidence of amyloid production. Unfortunately, he did not state whether these cells also stained positively with methylviolet or Congo-red. In the present study we observed some “grape cells” in the vicinity of “flame cells.” The “grape cells” stained positively with PAS but did not stain metachromatically with methyl violet, while the “flame cells” stained only faintly positively with PAS and metachromatically with methyl violet. It was previously shown21 that in rabbits immunized with killed typhoid bacilli a marked proliferation of pyroninophil plasma cells occurred. Many of these cells became transformed into “grape cells” and stained positively with PAS. It may be assumed that positive staining with PAS is a normal response to immunization and is not necessarily indicative of amyloid production. On the other hand, the appearance of “flame cells” staining faintly positively with PAS and metachromatically with methyl violet can be considered as evidence of intracellular amyloid production in plasma cells.

SUMMARY

Seventy W.-H. Swiss line mice were injected with adjuvant, TAB vaccine and TAB vaccine plus adjuvant. The 61 mice which survived form the subject of this study.
Spleen smears and histologic sections taken after completing immunization revealed a marked deposition of amyloid in 48 of the animals. Varying degrees of plasma cell hyperplasia were observed in 46 animals, and increased granulopoiesis in 37. In 28 of the amyloid-bearing animals some of the plasma cells were transformed into "flame cells" which stained faintly positively with PAS and metachromatically with methyl violet.

Electrophoresis of the pooled sera of animals receiving adjuvant alone, TAB plus adjuvant or TAB alone, compared to normal controls, showed decrease in albumine and an increase in the globulin fraction. Animals receiving adjuvant alone showed an increase in the beta globulins, while animals receiving TAB plus adjuvant or TAB alone showed an increase in the gamma globulins.

The relationship between the production of amyloid, plasma cell hyperplasia and the appearance of "flame cells" is discussed.

**REFERENCES**

5. Felix, A., and Anderson, E. S.: Immunizing potency of alcohol killed and alcohol preserved typhoid vac-

**SUMMARIO IN INTERLINGUA**

Septanta muses switze W, linea H recipeva injectiones de (1) adjuvante, (2) vaccino T.A.B., o (3) adjuvante e vaccino T.A.B. Le 61 superviventes es le base del presente studio.

Frottis splenic e sectiones histologic obtenite post le completion del immunisation revelava marcate depositos de amyloide in 48 del animales. Varie grados de hyperplasia plasmocytic eseva observate in 46 del animales e augmentos de granulopoiese in 37. In 28 del animales con amyloide, alcunes del plasmocytos eseva transformate in "cellulas flammante" que reageva con leve positivitate in tincturation con acido periodic de Schiff. Con violeto methylic lor reaction eseva metachromatic.

Le studio electrophoretic de specimens de sero collective ab le animales in cata un del tres gruppos de tractamento mostrava, in comparation con normal animales de controlo, un reduction del fraction de albumina e un augmento del fraction de globulina. Le animales tractate con solmente le adjuvante mostrava un augmento del globulinas beta, durante que le animales recipiente vaccino T.A.B. e adjuvante o solmente vaccino T.A.B. mostrava un augmento del globulinas gamma.

Es discutite le relation inter le production de amyloide, le occurrence de hyperplasia plasmocytic, e le apparition de "cellulas flammante".
Studies on the Fibrinolytic System in Cats Following Splenectomy.


Experiments were conducted on 11 cats. It was found that splenectomy did not induce any distinct changes in fibrinolysis levels nor in the process of fibrinolysis activation in total plasma. Inhibition of fibrinolytic activity was observed in euglobulins, as well as a decrease of the plasminogen level and an increase of the antiplasmin level. The induction of heterologous hemolytic shock in splenectomized animals did not yield the activation of fibrinolytic activity in euglobulins observed in intact animals.—E. K.
The Cytologic Reaction of the Spleen in Experimental Amyloidosis in Mice

AVINOAM ZLOTNICK and CHLOE TAL