STUDIES ON THE RESORPTION OF EXPERIMENTAL AMYLOIDOSIS

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SUMMARY.—The resorption of experimental murine amyloidosis induced by Freund’s adjuvant was studied over a period of 45 weeks. Three weeks after cessation of the injections splenic amyloid was found in 56-4 per cent of the animals and hepatic amyloid in 10-3 per cent of the animals. Thereafter the incidence of amyloid decreased steadily by the 20th week but splenic amyloid was still present in 14-3 per cent of the animals after 45 weeks. Resorption of hepatic amyloid paralleled that of the splenic amyloid. Renal amyloidosis was not striking and did not increase during the resorption of splenic amyloid. The morphological picture encountered during resorption included alteration in the specific staining qualities of the amyloid in the later stages, an increase in the number of giant cells, lymphoid hyperplasia and infiltration of amyloid by histiocytes and polymorphonuclear leucocytes. The significance of these histological findings in relation to the process of resorption is discussed. No spontaneous amyloidosis was found.

Some clinical observations have indicated that human amyloid may be reabsorbed and even disappear (Waldenstrom, 1928; Rosenblatt, 1936; Grayzel and Jacob, 1938). However, most of the cases were studied without the assistance of biopsies and were not subject to controlled studies. In fact, evidence for true regression of human amyloid is sparse (Parkins and Bywaters, 1959; Brandt, Cathcart and Cohen, 1968). Experimental amyloid formation has been extensively studied (Cohen, 1965; Ranløv, 1967; Jannigan and Druet, 1968) but relatively little attention has been paid to the process of resorption of experimental amyloid. Nevertheless, the few experimental studies performed suggest that amyloid is reabsorbed (Dick and Leiter, 1941; Richter, 1954; Williams, 1967).

The present investigation was intended to be a long-term follow-up study of the process and mechanism of resorption of experimental murine amyloid in the spleen, liver and kidneys. It was also anticipated that the present study would throw light on the role of the reticulo-endothelial system and enzymatic processes during resorption.

MATERIALS AND METHODS

A total of 500 male albino mice of local stock, aged 6 weeks and weighing 20–25 g., were injected i.m. with 0-3 ml. of complete Freund’s adjuvant, once a week for 6 weeks. The Freund’s adjuvant was prepared as in previous studies, using Mycobacterium tuberculosis (Tal and Laufer, 1960).

Throughout the experiment a total of 52 animals died of intercurrent infection, and were excluded from the study. Fourteen days after cessation of the injections 92 animals (group 1) were killed and a week later 39 animals (group 2) were killed in order to assess the incidence of amyloid in animals 2 and 3 weeks after the cessation of the injections. The remaining
animals were divided into groups of 40 and killed 5, 9, 12, 16, 20, 28, 36 and 45 weeks after the cessation of the injections (groups 3–10). Group 11 consisted of 20 animals which were kept alive until the end of the experiment but did not receive Freund’s adjuvant at all. This group served as a control group to assess the incidence of spontaneous amyloidosis and the effect of aging during the course of the experiment. The organs of these animals also served as controls for comparing morphology in treated and non-treated animals.

All animals were fed Purina Lab Chow and tap water ad libitum and were housed 20 per cage. It was decided not to perform serial follow-up biopsies on the animals for a number of reasons. It is known that amyloid may be focal and random biopsy of the spleen is not representative. Furthermore, repeated biopsies over a period of a year would have resulted in a high morbidity and mortality rate, and the histology would have been complicated by inflammatory reactions in the region of the biopsies.

After death, the spleens, livers and kidneys of all animals were fixed in 4 per cent formalin and stained with haematoxylin and eosin and a large number of special stains including methyl green pyronine, colloidal iron, alcian blue, van Gieson, periodic acid Schiff (PAS), mucicarmine, Congo red, gentian violet, and thioflavine T were performed. Sections stained with Congo red were examined under polarized light and those stained with thioflavine were examined with a fluorescent microscope using blue and ultra-violet excitation. Selected sections were stained with Prussian blue in order to assess the degree of hemosiderosis present. Other sections were treated with hyaluronidase and trypsin and then restained with Congo red and colloidal iron.

RESULTS

The results of the experiment in relation to splenic amyloid are summarized in the Table and represented together with the incidence of hepatic and renal amyloid graphically in Fig. 1. For the sake of brevity, only the findings in the organs during the stage of resorption will be described. Findings in the pre-amyloid and amyloid stages were similar to those encountered in previous studies (Lauffer, Tal and Behar, 1959; Lauffer, Tal and Kolander, 1968). The extent of amyloid when present was graded from + to +++++ according to microscopical findings (+ mild, ++ moderate, +++ marked, ++++ very marked).

The amyloid was present mainly perifollicularly, along the splenic sinusoids, and only rarely involved small arteries. It appeared as an eosinophilic amorphous material which showed characteristic staining qualities. Staining with Congo red was always positive and typical apple-green birefringence was elicited under polarized light. The gentian violet was not always metachromatic, but

**TABLE.—Summary of Incidence and Degree of Splenic Amyloid in Mice Killed from 2–45 Weeks after Completion of 6 Weekly Injections of Freund’s Adjuvant**

<table>
<thead>
<tr>
<th>Group</th>
<th>Time after injection (weeks)</th>
<th>Number of animals</th>
<th>With splenic amyloid*</th>
<th>Amyloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>92</td>
<td>28 (30-4)</td>
<td>++++</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>39</td>
<td>22 (36-4)</td>
<td>++++</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>33</td>
<td>9 (27-3)</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>39</td>
<td>6 (15-4)</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>39</td>
<td>13 (33-3)</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>37</td>
<td>2 (5-4)</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>32</td>
<td>4 (12-5)</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>34</td>
<td>4 (11-8)</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>35</td>
<td>4 (11-4)</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>28</td>
<td>4 (14-3)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Figures in brackets are per cent.
there was typical fluorescence after staining with thioflavine T. PAS, colloidal iron and alcian blue stains were in most cases conclusively positive. Amyloid, when stained with van Gieson, appeared khaki in colour. Digestion with hyaluronidase and trypsin had no effect on the amyloid, which stained positively after the use of these enzymes.

**Spleens during resorption.**—Spleens in the groups of animals with a low incidence of amyloid (i.e. particularly groups 6–10) frequently showed striking hyperplasia of the lymphoid follicles which were often confluent (Fig. 2). These hyperplastic follicles were surrounded by a wide rim of mononuclear and histiocytic cells, interspersed occasionally with scantily deposited amyloid. In general, little amyloid was found in these animals and when present it was ++ to +++ in degree and distributed perifollicularly. The red pulp showed a striking increase in the number of multinucleated giant cells (Fig. 3). Often these cells were found in the vicinity of amyloid or eosinophilic amorphous material which did not have the staining properties of true amyloid. The cytoplasm of these giant cells often merged into these masses but generally did not take up any of the specific stains for amyloid (Fig. 4). In addition, there were far less plasma cells, eosinophils and PAS positive mononuclear cells present in the red pulp. Occasionally there was diffuse infiltration of the pulp by polymorphonuclear leucocytes and some-

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**EXPLANATION OF PLATES**

**Fig. 2.**—Striking hyperplasia and confluence of lymphoid follicles in the spleen. H. and E. × 13.

**Fig. 3.**—Increased number of giant cells in red pulp of the spleen. Note irregular, ill defined eosinophilic cytoplasm. H. and E. × 130.

**Fig. 4.**—Giant cells and mononuclear cells adjacent to amyloid masses in the spleen. H. and E. × 130.

**Fig. 5.**—Focus of hepatic amyloid infiltrated by histiocytes. H. and E. × 130.

**Fig. 6.**—Focus of hepatic amyloid infiltrated by histiocytes and polymorphonuclear leucocytes. H. and E. × 130.

**Fig. 7.**—Glomerular amyloidosis. H. and E. × 13.
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times focal aggregates of polymorphs were present adjacent to masses of amyloid. Hemosiderosis was a constant feature and the iron was found both in phagocytes and extracellularly. In the later groups (7–10) many of the spleens showed an almost normal architecture, however lymphoid hyperplasia and an increased number of giant cells were present.

The amyloid retained most of its characteristic staining qualities throughout the process of resorption and changes in tinctorial properties were found in groups 7–10 but particularly in groups 9 and 10. These were mainly expressed by negative colloidal iron staining, less striking dichroism under polarized light and at times by absent fluorescence with thioflavine T. Digestion studies using trypsin and hyaluronidase were inconclusive. The spleens of group 11 (controls not receiving Freund’s adjuvant) showed an occasional increase in the number of giant cells but no spontaneous amyloid was encountered.

Liver.—Changes were predominantly focal, not present in all animals, and generally little amyloid was encountered. There was no characteristic picture of resorption recognizable in this organ. However, it is worthy of note that in some of the animals of groups 9 and 10 foci of amyloid which had retained some of its tinctorial properties were infiltrated by large numbers of histiocytes and leucocytes (Figs. 5 and 6). The amyloid present was Congo red positive but showed negative dichroism with polarized light and stained negatively with thioflavine T. and colloidal iron. Most livers showed a normal architecture, however in some animals there were foci of periportal and parenchymal inflammation, recent parenchymal necrosis infiltrated with leucocytes and signs of hepatocyte regeneration.

Kidney.—Only a very small number of animals showed renal amyloidosis and when amyloid was present it was only glomerular (Fig. 7). No peritubular and vascular involvement was encountered, and there was no glomerular fibrosis. No characteristic picture of resorption was present.

Reaction to Freund’s adjuvant.—This was present in the local subcutaneous tissues in the region of the injection, in the capsule of the spleen and liver and in the peripancreatic fat of most animals, as described in other studies (Laufer et al., 1959; Laufer, Rosenmann and Davies, 1966). This finding was encountered in all groups of animals examined and it bore no relation to the distribution and extent of amyloid deposition in the different organs. This reaction to the adjuvant was even found in animals of groups 7–10, without amyloidosis.

Amyloid production in both the spleen and liver reached an incidence of 56.4 and 10.3 per cent respectively, 3 weeks after cessation of injections with Freund’s adjuvant. The amyloid deposited in the spleens of these animals was predominantly perifollicular and in a little less than half of the animals was of a marked degree. After 3 weeks, there was a steady decline in the incidence of amyloid and in the groups killed after 16 weeks, the degree of amyloidosis in the spleen was always mild to moderate. The incidence of amyloid in these groups was significantly lower statistically when compared to group 2 (according to the Mann-Whitney U test \( Z = 4.48, P < 0.0001 \)).

DISCUSSION

The results of this study show that resorption of experimental amyloid occurs. However, amyloid did not disappear entirely from all the organs and splenic amyloid was still present in 14.3 per cent of the animals after 45 weeks.
There was a peak of amyloid formation encountered in animals killed 3 months after cessation of the injections (group 5) and the incidence of splenic amyloid rose to 33-3 per cent and hepatic amyloid to 17-9 per cent. This second peak of amyloidogenesis was unexpected and may have been related to a sudden release of adjuvant from the site of injection. However, its cause and significance remain obscure. This peak was not of statistical significance when compared to group 4 ($X_1^2 = 3.41$) and was of minimal significance in relation to group 6 (Yate’s correction $X_1^2 = 7.67, P < 0.01$).

In the present study there appeared to be a reasonably typical morphological picture during the resorption of splenic amyloid. We noted a definite increase in the number of giant cells throughout the process of resorption. However, it was difficult to demonstrate phagocytosis of amyloid by these cells despite the fact that many of them appeared in close relation to the amyloid. An increased number of giant cells has also been encountered in other studies on the resorption of experimental amyloid (Richter, 1954). However, their relation to the process of resorption is still controversial (Dick and Leiter, 1941; Williams, 1967). Giant cells are occasionally present in the region of human amyloidosis, but whether they are indicative of a resorptive process is also unclear.

Other features of resorption were the presence of hemosiderosis, the small numbers of pyroninophilic plasma cells and eosinophils, and the formation of large, often confluent, lymphoid follicles. This finding has also been recorded in other studies (Richter, 1954; Williams, 1967) and was predominant in the spleen in Williams’ study. In contrast to these findings, Dick and Leiter (1941) recorded atrophy of lymphoid follicles during resorption. The follicles were often surrounded by a wide rim of mononuclear and histiocytic cells, intermingled at times with amyloid present in this area. It is possible that these cells are in some way responsible for the resorption of the amyloid, in the later stages of the experiment.

Occasionally, polymorphonuclear leucocytic infiltration was seen in the spleen, sometimes in close relation to masses of amyloid. This was also encountered focally in the liver, in a more striking fashion, but the amyloid, present in these areas, did not always retain its tinctorial qualities. Generally, hepatic amyloid was never striking and was always focal and its incidence and resorption appeared to parallel that of splenic amyloid. In our study, as in Dick and Leiter’s study, there was little conclusive evidence other than that described above for the resorption of hepatic amyloid.

In some studies (Dick and Leiter, 1941) the incidence of renal amyloid reached a peak incidence of approximately 70 per cent towards the end of the study. In other studies (Williams, 1967) renal amyloid was severe, appeared irreversible and was often present while splenic amyloid was still prominent. In contrast to these findings, there was no increase in the incidence of renal amyloid in the late stages of our experiment. Amyloid, when present in the kidneys, was scantily distributed, glomerular and rarely in the small vessel wall, and was encountered only a very small number of animals. Renal amyloid did not increase concomitantly with a reduction of splenic amyloid and thus it appears unlikely that its presence was due to mobilization of splenic amyloid and it is more likely to be due to local formation.

It is of interest to note that generally the amyloid retained its tinctorial properties throughout the course of the experiment and only after 20 weeks was
an alteration in tinctorial quality noted. This was more striking at 36 and 45 weeks and may be due to aging of the amyloid, as suggested by some workers (Battaglia and Matturri, 1965), or to enzymatic digestion by leucocytes or giant cells as an integral part of the process of resorption.

It is of importance to note that our study of amyloid resorption was performed in mice and that most of the studies discussed above were performed in rabbits (Dick and Leiter, 1941; Richter, 1954; Williams, 1967). It is therefore possible that the late rise in the incidence of renal amyloid and some of the morphological differences encountered in the different studies may be related to the difference in species and experimental technique used to produce amyloid in the various studies. This may be an additional factor complicating the interpretation and comparison of the various findings encountered in the different studies.

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REFERENCES