Experimental Production of “Grape Cells” and Their Relation to the Serum Gamma Globulin and Seromucoids

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It has been suggested in a previous publication that the presence of intracytoplasmic inclusions in bone marrow plasma cells, in the form of “morular cells,” “grape cells” and crystals, is associated with hyperglobulinemia and a concomitant plasma cell hyperplasia. From the work of Bing and Plum, Bjørneboe and Gormsen, Fagreus and Moeschlin, it is evident that the process of immunization manifests itself by a pronounced proliferation of plasma cells, especially in the spleen, and by an increase of the serum gamma globulin.

The purpose of this study was to try to produce grape cells, to determine whether their presence was associated with an elevation of the serum gamma globulin and to identify the nature of their globules by cytochemical methods. As the grape cells stained positive with periodic acid Schiff, thus indicating the presence of a probable polysaccharide, we also tried to find out whether there was a correlation between the presence of grape cells and the level of seromucoids in the serum.

Material and Methods

Thirty rabbits weighing approximately two Kg. each were immunized for seven weeks with three different antigens. Two rabbits were injected with normal saline and used as controls.

One group of animals was immunized with a saline suspension of alcohol-killed, alcohol-preserved, typhoid bacilli injected intravenously three times weekly in increasing doses of 0.25 ml. A total of approximately 150 billion bacilli was injected.

A second group was immunized with a smallpox vaccine prepared from material collected from vaccinal lesions on the skin of calves, diluted with saline to a concentration of 1:10. Subcutaneous injections were administered thrice weekly for seven weeks in increasing doses of 0.25 ml until a maximum dose of 4 ml per injection was reached.

The third group of rabbits was immunized with antirabies vaccine; a suspension of 5 per cent to 10 per cent of infected rabbits' brains inactivated in 1 per cent phenol was used. Subcutaneous injections were administered thrice weekly for seven weeks in increasing doses of 0.5 ml until a maximum dose of 6 ml per injection was reached.

Blood samples were taken and a splenic biopsy was performed under local anesthesia two to seven days prior to the administration of the antigen and two to seven days after the last injection. Total serum proteins, their electrophoretic pattern—obtained by

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Table 1.—Electrophoretic Pattern of Serum Proteins and Seromucoid Level and Their Relation to the Presence of Grape Cells Before and After Seven Weeks of Immunization with Three Different Antigens

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Number of animals</th>
<th>Total serum proteins (mg%)</th>
<th>Albumin (%)</th>
<th>α globulin (%)</th>
<th>β globulin (%)</th>
<th>γ globulin (%)</th>
<th>Seromucoids (mg%)</th>
<th>Grape cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not immune</td>
<td>18</td>
<td>5.8</td>
<td>47.9</td>
<td>13.8</td>
<td>18.8</td>
<td>19.5</td>
<td>58.6</td>
<td>0</td>
</tr>
<tr>
<td>Saline</td>
<td>2</td>
<td>5.8</td>
<td>48.6</td>
<td>16.3</td>
<td>15.4</td>
<td>19.7</td>
<td>57.0</td>
<td>0</td>
</tr>
<tr>
<td>Typhoid</td>
<td>10</td>
<td>6.5(6)</td>
<td>45.5(6)</td>
<td>14.4(6)</td>
<td>12.6(6)</td>
<td>27.5(6)</td>
<td>102.8(12)</td>
<td>+9</td>
</tr>
<tr>
<td>Smallpox</td>
<td>6</td>
<td>6.9</td>
<td>35.7</td>
<td>14.2</td>
<td>18.9</td>
<td>31.2</td>
<td>112.0(1)</td>
<td>+6</td>
</tr>
<tr>
<td>Rabies</td>
<td>4</td>
<td>7.2(6)</td>
<td>33.3(6)</td>
<td>14.5(6)</td>
<td>22.4(6)</td>
<td>29.2(6)</td>
<td>130.0(4)</td>
<td>+4</td>
</tr>
</tbody>
</table>

The italic numbers within ( ) indicate the number of animals examined. The numbers in the last column indicate the number of animals in which grape cells were found.

Filter paper electrophoresis—and the serum seromucoids—were determined before and after immunization. Smears were prepared from the splenic material, dried and stained with the May-Grünwald-Giemsa combination. Smears found to contain many intracytoplasmic inclusions in the plasma cells were stained with PAS,7 PAS after digestion with saliva, Sudan Black B,9 toluidine blue,10 Bicol,11 Methyl green pyronin12 and osmium tetroxide.13 At least two smears were examined for half an hour each before a result was recorded as negative.

RESULTS

Of the 32 animals initially selected for this study, 10 died before the end of the experiment. The data summarized hereafter concern the 22 surviving animals after the seven weeks' experiment.

Table 1 indicates the results of the serum analysis for total serum proteins, paper electrophoresis and seromucoids in 18 not immunized rabbits, in 14 immunized animals and in two controls injected with normal saline. In none of the not immunized animals were grape cells found. Only in one rabbit—which was not used subsequently in the experiment—did we find some grape cells; this rabbit had a broken leg. The electrophoreses examined in two out of four rabbits immunized with antirabies vaccine and in six rabbits immunized with smallpox showed an increase in the total proteins, a decrease of the albumin fraction and an elevation of the gamma globulin fraction. In all these immunized animals grape cells were found in smears of the spleen after completing the immunization. Two control animals injected with normal saline did not show any significant variation from the normal.

Ten rabbits were immunized with killed typhoid bacilli. In only six of these were total proteins and electrophoreses determined. Here, too, there was an increase in the total proteins and in the gamma globulin fraction. However, in contrast to the results obtained with the other antigens, there was no decrease in the albumin fraction but a significant fall in the beta globulin fraction. Grape cells were found in nine out of the 10 immunized animals.

The seromucoid fraction of the serum was determined in eighteen not immunized rabbits, in 10 animals immunized for seven weeks with killed typhoid bacilli, in one rabbit immunized with smallpox, in one rabbit im-
munized with antirabies vaccine and in two controls injected with normal saline. In all the immunized rabbits there was a marked rise in the seromucoid fraction, which corresponded to the increase in the gamma globulin fraction. The rabbits injected with saline did not show any rise in the seromucoid fraction.

In all splenic smears of these hyperimmune animals there was a marked proliferation of mature and immature plasma cells as compared with the smears of the same animals before immunization. There were, however, marked individual differences. In some of the animals, the number of plasma cells after immunization was not much above the number found before immunization in other animals. In animals showing a marked number of plasma cells before immunization, the response to immunization manifested itself by a conspicuous plasma cell hyperplasia.

As already mentioned, grape cells were found in all but one of the hyperimmune rabbits. In some they appeared after three weeks of immunization. Their number varied from animal to animal and in the same smear from area to area. There were areas containing many cells and areas devoid of them. In all animals the number of grape cells corresponded to the degree of plasma cell hyperplasia, the higher the latter, the greater the number of grape cells.

The typical grape cell is a plasma cell, the cytoplasm of which contains round vesicles staining lightly gray-blue with the May-Grünwald-Giemsa method. They vary in number; when they are rare the blue cytoplasm between the vesicles can be distinguished (fig. 1); when their number increases the cytoplasm is not seen and the cell's limits are blurred (fig. 2). The nucleus is sometimes pyknotic. The vesicles can be found in young plasma cells as well as in mature ones (fig. 3).

In some of the animals we found, in addition to the grape cells, plasma cells containing cuboid crystals (fig. 4) and plasmacytoid reticulum cells containing some material in the form of granules or tiny needles arranged in different directions (fig. 5).

Histochemical studies revealed that the globules of the grape cells and the other intracytoplasmic inclusions of the plasma cells, gave a positive reaction with PAS, with considerable variation in staining intensity, ranging from pale pink to deep red (fig. 6). Exposure of the smears to the action of saliva did not change the positive reaction with PAS. The globules gave a negative result for fats with Sudan Black B. Anisotropism was absent. The Toluidine blue reaction for metachromasia and the Bicol reaction of Wolman$^{12}$ for differentiation of strongly acid groups from weakly acid ones, were negative. The globules of the grape cells stained brown with Osmium tetroxide dissolved in a non-polar solution, but did not stain with Osmium tetroxide in aqueous solution, which, according to Wolman,$^{13}$ is probably indicative of the presence of glucosamine. With methyl green pyronin the grape cells did not stain, while the plasma cells took on a deep red color, thus indicating the absence of ribonucleic acid in the vesicles of the grape cells.
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**Fig. 1.** Young "grape cell": mature plasma cell containing a few globular bodies. The limits of the cell are preserved. The blue cytoplasm can be seen between the globules. (Typhoid; × 1000)

**Fig. 2.** Mature "grape cell": The plasma cell can hardly be recognized. The nucleus is eccentric. The globules are numerous and press together, assuming a polyhedral shape. The limits of the cell are destroyed. (Typhoid; × 1000)

**Fig. 3.** Young plasma cell containing many globular bodies. (Typhoid; × 1000)

**Fig. 4.** Plasma cell containing cuboid crystals. (Typhoid; × 1000)

**Fig. 5.** Plasmacytoid reticulum cell with two nuclei, containing needle-like inclusions arranged in different directions. (Typhoid; × 1000)

**Fig. 6.** Positive stain of a "grape cell" with PAS. (Rabies; × 1000)
The presence of grape cells in the bone marrow has been found to be associated with hyperglobulinemia and plasma cell hyperplasia. As the reticuloendothelial system responds to a variety of foreign antigens with a marked proliferation of plasma cells and an elevation of the serum gamma globulin fraction, it was of interest to find these cells in spleens of hyperimmunized rabbits presenting also hypergammaglobulinemia and plasma cell hyperplasia.

The globules of the grape cells and the other inclusions of the plasma cells which stained gray-blue with the May-Grönnwald-Giemsa combination gave a positive reaction with periodic acid Schiff (PAS). As the absence of lipids had been verified by the negative result with Sudan Black B, the polysaccharide nature of these globules was thus established. Digestion with saliva did not change the PAS reaction, indicating that this polysaccharide was not glycogen. The positive reaction for glucosamine and the negative reactions for metachromasia and for free acid groupings would suggest that the substance is a neutral mucoprotein. Similar observations were reported by White and Teilum, who produced by hyperimmunization eosinophilic Russell bodies and granular reticuloendothelial cells which were PAS-positive.

The relationship between the grape cells and the seromucoids was studied. This revealed that grape cells appeared three weeks after the beginning of immunization, their appearance coinciding with the maximum increase in the level of the serum seromucoids. The number of grape cells continued to increase even though there was, later on, a decline in the level of the seromucoids. From our data as well as from those of Weimer, it is evident that the process of immunization is associated with an increase in the level of the seromucoids in addition to an elevation of the gamma globulin and the plasma cell hyperplasia.

The seromucoid level is known to be high in patients suffering from carcinoma, myocardial infarction, infections, as well as after experimental irradiation. It is assumed that this is caused by destruction of the tissue ground substance which contains mucoproteins, thus releasing the seromucoids in the blood. As an alternative explanation for this elevation of the seromucoids in carcinoma, it might be suggested that a process of autoimmunization through abnormal proteins produced by the tumor takes place. In support of this suggestion we may bring forth the observation of Pears that around malignant tumors there is a marked proliferation of plasma cells and PAS-positive Russell bodies, indicating an increased production of mucoproteins. A similar observation was made recently by Rappaport et al., who in a study of patients with malignant lymphomas associated with hemolytic anemia found in the neoplastic lymphocytes a PAS-positive intracellular substance, presumably a mucoprotein. This is in accordance with Dameshek et al., who suggested that malignant lymphomas may be the site of production of abnormal proteins—possibly antibodies—which cause hemolytic anemia. We may, therefore, assume that there exists a re-
Relationship between the process of immunization, the production of mucoproteins and the presence of grape cells.

In our previous publication, confirmed by Rondanelli et al., the grape cells found in bone marrow were described as PAS-negative. This difference might be due to the fact that in our previous work the material used was taken from patients suffering from hepatic cirrhosis, in whom the seromucoid content was below normal. It may be assumed, therefore, that the staining behavior of the substance of the grape cell differs according to the seromucoid level of the serum.

SUMMARY

Hyperimmunization in rabbits with three different antigens produced an elevation of the serum gamma globulin, a plasma cell proliferation and an increase of the seromucoid content of the serum.

Grape cells and intracellular crystals were found in hyperimmune animals, but not in controls. The globules of the grape cells stained positive with periodic acid Schiff and with osmium tetroxide dissolved in a non-polar solution, indicating the presence of a mucopolysaccharide.

It is suggested that there is a relationship between the process of immunization—producing hyperglobinemia, plasma cell hyperplasia and an elevation of the serum seromucoid content—and the presence of grape cells.

REFERENCES

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