Production of Nucleophagocytosis by Rabbit Antileukocytic Serum

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The factor responsible for the "L.E." phenomenon in patients with systemic lupus erythematosus has been found to be associated with the gamma globulin fraction of the plasma. It has been suggested that the "L.E." phenomenon represents an immunologic mechanism. Although the plasma "L.E." factor has been shown to be antigenic, this does not explain the mechanism of the "L.E." phenomenon. This phenomenon includes the ingestion of nuclear material from autolyzed leukocytes by other polymorphonuclear leukocytes. The supposition that the "L.E." phenomenon may be due to an antibody led the authors to attempt to reproduce this phenomenon by producing an antileukocytic serum.

Method and Procedure

The sensitizing material was obtained from a patient with granulocytic leukemia with 450,000 cells of the granulocytic series per cu.mm. of blood. The buffy coat layer of a centrifuged specimen of blood from this patient was allowed to dry overnight and was thoroughly ground. One Gm. of this dried material was suspended in 100 cc. of isotonic saline. This crude suspension was injected subcutaneously into a rabbit at three day intervals for the following eight weeks. The first dose was 0.5 cc. and all subsequent doses were 1 cc. Plasma obtained from the rabbit was studied for its ability to reproduce the "L.E." phenomenon at approximately four week intervals after the initiation of this sensitization. This was done by mixing the buffy coat layer of the peripheral blood of the patient who was the source of the antigen with the rabbit plasma, utilizing the method of Haserick and Bortz.

Results

The plasma of the rabbit was first tested for its ability to produce the "L.E." phenomenon four weeks after sensitization was begun. In this preparation there was abundant evidence of nucleophagocytosis. Many of the engulfed nuclei showed clear chromatin patterns and were similar to the "tart cell" of Hargraves. In almost all cases, however, the engulfing cell was a polymorphonuclear neutrophil rather than a histiocyte. Furthermore, many of the engulfed nuclei showed loss of chromatin pattern to a varying degree (fig. 1 a–e). In some, this resulted in a homogeneous inclusion body (fig. 1e), producing a cell resembling the "L.E." cell. Cells with varying degrees of karyorrhexis of the nuclei, resembling the "pre-L.E." cell described by Stitch, were also seen (fig. 1f). Numerous clumps of polymorphonuclear leukocytes were observed in this preparation (fig. 1c).
In some instances, apparent nuclear material with varying degrees of chromatin pattern occupied a central position in the clump. A specimen of plasma obtained at twelve weeks revealed the same phenomenon but with only an occasional cell showing nucleophagoctosis. This, however, was associated with marked

Fig. 1.—Nucleophagoctyes (a–e), clumping of leukocytes (e), and karyorrhexis (f).
fragmentation and destruction of leukocytes, with relatively few intact polymorphonuclear neutrophils seen in the entire preparation. In addition, numerous free homogeneous purple-staining bodies were seen throughout the slide. Specimens of the rabbit plasma obtained at eight, sixteen, and twenty weeks failed to produce this phenomenon. There were, however, markedly fragmented and conglutinated polymorphonuclear leukocytes and occasional cells showing karyorrhexis. Occasional polymorphonuclear leukocytes containing engulfed erythrocytes were seen.

Throughout the period of observation, no adverse effects were shown by the rabbit. The coat remained glossy and there was no evidence of physical deterioration. Examination of theuffy coat layer of rabbit blood revealed none of the phenomena described above. Likewise, repeated examination of specimens of heparinized bone marrow and peripheral blood buffy coat layer from the donor patient failed to reveal any of the changes produced by the rabbit antileukocytic serum.

**DISCUSSION**

Mixing antileukocytic serum with leukocytes from the same source as the antigen resulted in nucleophagocytosis, karyorrhexis, and clumping of polymorphonuclear leukocytes. Prior to sensitization, plasma from the rabbit failed to induce this phenomenon. Also, throughout the period of study, normal human serum failed to produce these changes in the indicator leukocytes. The ability of the rabbit plasma to induce nucleophagocytosis seems, therefore, a result of sensitization to leukocytes. The similarity of the resulting cells and the associated changes produced by antileukocytic serum to the “I.E.” phenomenon raises the possibility that the latter may also be due to an antileukocytic antibody.

A related study was reported by Cajano and Maurea in 1950. They studied the properties of antileukocytic sera produced in rabbits by sensitization to guinea pig bone marrow and leukocytes. The injection of such antisera into guinea pigs produced fragmentation of marrow elements and karyorrhexis of polymorphonuclear cells. Examination of the photographs in this study reveal a close similarity between these cells and the “pre-L.E.” cell of Stiteh.

The significance of the “tart” cell is obscure. Hargraves, who first described both the “L.E.” and the “tart” cell, emphasized the fact that the latter is usually a histiocyte while the former is almost always a neutrophilic polymorphonuclear leukocyte. Furthermore, this author states that the inclusion body of the “L.E.” cell is usually a homogeneous purple-staining mass with no visible chromatin pattern. When the “L.E.” cell contains an inclusion body that still shows a chromatin pattern, it can be differentiated from the “tart” cell, according to Hargraves, “by finding numerous ‘L.E.’ cells in which the process of digestion has altered the appearance of this chromatin material, ranging from small smoky remnants of ‘digested’ material to relatively intact nuclei indistinguishable from the secondary nucleus of the ‘tart’ cell.” Such transitions are seen frequently in preparations from patients with systemic lupus erythematosus. The nucleophagocytosis induced by antileukocytic serum was also characterized by forms transitional between distinct “tart” cells on the one hand, and cells whose in-
CLUSION bodies showed marked homogeneity on the other. The occurrence of such transitions suggests that the "L.E." cell and the polymorphonuclear "tart" cell may be related phenomena.

The marked conglutination and fragmentation of leukocytes produced by later specimens of rabbit serum may represent a higher level of the antibody responsible for the nucleophagocytosis. On the other hand, these changes may be due to different antibodies, as might be expected from so crude an antigen. The observations of Tischendorf and Fritze9 suggest that "aggregation" of leukocytes can occur independently of an antigen-antibody reaction. Further studies to throw light on these preliminary observations are planned.

SUMMARY

Dried buffy coat layer of the blood from a patient with granulocytic leukemia was injected into a rabbit. Serum from this rabbit, when mixed with the buffy coat layer of the blood from the same patient, resulted in the production of nucleophagocytosis. The serum of the rabbit also showed the ability to produce marked conglutination and destruction of the neutrophilic polymorphonuclear leukocytes.

REFERENCES

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