On the Interaction of Dead Leukocytic Nuclei, L.E. Factor and Living Leukocytes in the L.E. Cell Phenomenon

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In a previous communication we reported a simple procedure for the demonstration of L.E. cells from a single drop of peripheral blood from patients with systemic lupus erythematosus. This test has proved to be satisfactory in the work of others and has correlated well with other, more involved technics. This procedure is based on the use of a substrate slide containing dead, unfixed, normal or leukemic leukocytes which are brought into contact with a hanging drop of fresh suspect blood. From the resulting small clot large numbers of living leukocytes migrate toward the substrate slide. Due to the exudation of serum from the clot, L.E. factor is brought in contact with both dead and living leukocytes and results in the formation of large numbers of L.E. cells. From these and other studies we proposed that dead leukocytes were essential for L.E. cell formation. Only the nuclei of dead cells under influence of both L.E. serum and living leukocytes are transformed into the characteristic L.E. cell inclusion body. Using other technics, Zinkham and Conley later came to a similar conclusion.

In 1953 Miescher, using experimentally produced antinuclear serum, observed nucleophagocytosis and also the formation of cells highly reminiscent of those seen in lupus erythematosus. He obtained the best results by injecting guinea pigs with isolated nuclei obtained from human spleens and lymphocytes. When incubated with human leukocytes suspended in plasma, these antisera produced swelling of the nuclei of the polymorphonuclear cells, a tendency to stain red with Giemsa, and, finally, phagocytosis of the altered nuclear material by normal polymorphonuclear and mononuclear cells. In Miescher's opinion the cells resulting from this phagocytosis closely resemble the formation of classical L.E. cells. These experimental data suggested to him that the formation of L.E. cells under the influence of lupus serum depends upon the presence of an antinuclear auto-antibody. Miescher has added his belief that a low titer of this antibody results in the formation of Hargraves' "tart cells." When the titer increases, typical L.E. cells are formed, and with a further increase in the antibody concentration, "rosette" formation appears.

In subsequent work this investigator has shown that serum containing L.E. factor loses its activity when incubated with isolated leukocyte nuclei. In more recent experiments he has demonstrated that isolated cell nuclei, "sensitized" by the adsorption of L.E. factor, are phagocytized by viable leukocytes.

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This investigation was supported by a grant (No. H-3397) from the U.S. Public Health Service.
Submitted Oct. 7, 1957; accepted for publication Feb. 10, 1958.
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In our laboratory we also have been able to demonstrate the binding of L.E. factor to isolated leukocyte nuclei. The avidity of the nuclei for the lupus factor is so intense that the greater part of the later factor disappears from the lupus serum. Such "sensitized" nuclei when placed in contact with viable leukocytes are transformed into typical lupus bodies. The union between nuclei and lupus factor is prevented if the nuclei are contained within an intact cytoplasmic envelope. The role of the nuclei of dead leukocytes in L.E. cell formation is supported and clarified. These studies form the basis of this report.

**METHOD AND RESULTS**

_Preparation of Bare Nuclei_

Twenty cc. of oxalated* venous blood drawn from a normal person is mixed with 5 cc. of dextran solution† and allowed to settle in an inclined test tube until there is suitable separation of the buffy coat. The latter is removed and the leukocytes are separated from the plasma by centrifugation.§ The button containing leukocytes, platelets and few red cells is washed in 3 changes of 10 cc. of isotonic saline. After the final washing, all of the supernatant saline is removed. In order to separate the nuclei from the cytoplasm, the button is resuspended in 10 cc. of 0.2% citric acid§ and incubated for 30 minutes at 37 C. This tube is then centrifuged at 1000 r.p.m. for 5 minutes. The supernatant is removed and discarded. To remove the citric acid, the button is washed in 3 changes of 10 cc. isotonic saline by alternate centrifuging at 1000 r.p.m. for 5 minutes and resuspension. Giemsa stain of a sample of the resultant button reveals the leukocyte nuclei divested of cytoplasm (fig. 1). The red cells and platelets can no longer be identified.

*Testing Sera** for L.E. Cell Activity

All L.E. sera, before and after adsorption with intact leukocytes and bare nuclei, respectively, were tested for L.E. cell activity by the following method which has been used in this laboratory for the last 2 years:

1. Slightly modified substrate slides' in the form of thick smears of leukocytes obtained by centrifuging the buffy coat of oxalated blood are used. These smears are allowed to air-dry overnight before use, and for the best results remain suitable up to 7 days.

2. One drop of test serum, warmed to 37 C., and one drop of concentrated, fresh leukocytic buffy coat are placed on the approximate center of the previously prepared substrate slide. The 2 drops are mixed gently with the edge of a long cover glass and allowed to diffuse over the area of dead leukocytes by overlaying the cover glass directly on the slide. This preparation is then placed in a warm, humid petri dish chamber and incubated at 37 C. for 2 hours, as outlined in a previous publication.' The cover glass is then removed, the slide allowed to dry, fixed and stained with Giemsa.

L.E. cell activity of sera is reported as the number of L.E. cells observed per 100 or 500 leukocytes.

_Adsorption of L.E. Factor by Bare Nuclei_

The button of bare nuclei prepared as above is equally divided (accomplished easily before centrifugation of the last saline washing). Then, after centrifugation and decanting.

*The anticoagulant used throughout this study consisted of 6 mg. of ammonium oxalate and 4 mg. of potassium oxalate to each 5 cc. of whole blood.
†Abbott's Dextran 6% w/v in isotonic saline.
§Buffy coats, whenever referred to in this paper, are prepared in this manner.
**Serum should be stored aseptically at 4 C. because repeated deep-freezing and thawing reduces L.E. cell activity.
Fig. 1.—Isolated leukocyte nuclei prepared by treating leukocytes with 0.2% citric acid.

½ of the button is suspended in 2 cc. of serum from a patient with potent L.E. cell activity, the other half in 2 cc. of normal serum. Both suspensions are incubated at 37 C. for 1 hour. The leukocytic nuclei are then separated from the serum by centrifugation. The supernatant serum of the button suspended in L.E. serum is saved for determination of residual L.E. cell activity. The supernatant serum from the control suspension is discarded. Both buttons are then washed by vigorous resuspension in 10 cc. of cold isotonic saline, centrifuging at 1000 r.p.m. for 5 minutes and decanting. This is repeated 3 times.

Each of the 2 buttons is then resuspended in 5 cc. of freshly prepared, unmodifieduffy coat containing viable leukocytes. Thisuffy coat is prepared from the same person who was used as the source for the bare nuclei. These preparations are then incubated for 2 hours at 37 C., centrifuged and Giemsa stained smears made from the buttons.

Ten experiments conducted with 2 potent L.E. sera revealed that intense L.E. cell
formation, rosettes and globs were obtained in each instance from incubating viable leukocytes with such washed, leukocytic nuclei which previously had been incubated with serum containing L.E. factor. Approximately 50 L.E. cells were seen per low power field (x128) (fig. 2). In a similar number of control experiments using leukocytic nuclei incubated with normal serum, no semblance of the L.E. phenomenon was observed.

The adsorption of L.E. factor to leukocytic nuclei is remarkably tenacious; the intensity of L.E. cell formation was not affected by washing the nuclei after adsorption in as many as 10 changes of cold isotonic saline or distilled water. The L.E. cells possessed the typical swollen, structureless inclusion body as described by Hargraves and were not the products of simple nucleophagocytosis (fig. 2).

In all instances the supernatant sera after adsorption with bare nuclei revealed striking
reduction in activity. Whereas nonadsorbed, control sera resulted in the formation of approximately 75 to 100 L.E. cells per 100 leukocytes in all trials (fig. 3), only a trace of activity, i.e. one L.E. cell per 500 leukocytes (fig. 4), remained in the L.E. sera which had been in contact with bare nuclei. Re-incubation of such sera with a fresh batch of bare nuclei removed the remaining L.E. cell activity. In a few instances the initial adsorption was sufficient to remove all demonstrable activity.

"Adsorption" of L.E. Factor by Intact, One-Day-Old Leukocytes

It next was decided to determine the adsorbing properties of the intact leukocyte. A suspension of freshly drawn leukocytes could not be used. Because of the death of part

Fig. 3.—Intense L.E. cell formation by the control L.E. serum. Here, a mixture of viable leukocytes and nonadsorbed L.E. serum is placed in contact with a substrate of dead, intact leukocytes.
Fig. 4.—When the same experiment is repeated using the supernatant L.E. serum after adsorption with isolated nuclei, the L.E. cell activity is reduced to a minimum.

of the white cells during the preparation of such a suspension, the latter would represent a mixture of dead and living leukocytes. Incubation of this mixture with L.E. serum would result in the formation of large numbers of L.E. cells. The freshly drawn leukocytes were therefore kept for a 24-hour period outside the body to produce cellular death. While the majority of the cells remain structurally intact at the end of this time, beyond this period considerable cellular deterioration occurs.

In this experiment a buffy coat is first kept at room temperature for 24 hours, during which time the majority of the leukocytes die. The leukocytes are then separated from the plasma, washed 3 times with isotonic saline, incubated with L.E. serum and again washed as outlined above for bare nuclei. The supernatant serum is saved for the determination of residual cell activity. Before the addition of the fresh buffy coat to the button of
“adsorbed” one-day-old intact leukocytes, one drop is smeared on a glass slide and examined for L.E. cells. This serves as a control to determine whether some one-day-old leukocytes, despite such vigorous treatment, remained sufficiently viable to form L.E. cells directly from the dead leukocytes present in the suspension. This proved to be true since small numbers of L.E. cells were found. In these preparations approximately one L.E. cell per low power field (x128) was seen in 5 similarly conducted experiments.

The addition of 5 cc. of fresh buffy coat to the remainder of the button of “adsorbed” one-day-old leukocytes in each of 5 trials revealed no significant further increase in the number of L.E. cells formed after 2 hours incubation at 37 C. (fig. 5).

When the supernatant L.E. sera after adsorption with intact one-day-old leukocytes

Fig. 5.—Intact, one-day-old leukocytes after “adsorption” with L.E. serum and subsequent incubation with viable leukocytes yield only slight L.E. cell formation. The arrow points to an L.E. cell.
were examined, there was no demonstrable diminution in L.E. cell activity in any instance. Here, as existed with nonadsorbed control sera, approximately 75 or more L.E. cells per 100 leukocytes were seen in all preparations (fig. 6).

DISCUSSION

The present study, confirming Miescher’s experiments⁶,⁷ demonstrates the affinity of L.E. factor for leukocytic nuclei and further indicates the blocking effect of the surrounding cytoplasmic material.

If one accepts Miescher’s concept that the L.E. factor is an antinuclear

Fig. 6.—The supernatant L.E. serum after incubation with intact one-day-old leukocytes reveals no appreciable diminution in L.E. cell activity.
auto-antibody, without immunologic relationship to cell cytoplasm, the results of these experiments are easily explained. Since the nucleic acid moiety of the nucleoprotein complex residing in the nucleus differs chemically from cytoplasmic nucleic acid (i.e. in the pentose and pyrimidine components), it is quite probable that the parent nucleoproteins have different antigenic properties. Dissimilar structure of the protein moiety (histone) may also be responsible for the immunologic specificity of the cell nucleus.

It appears unlikely that the cytoplasm exerts its inhibitive action by a mechanism of biologic competition. The failure to demonstrate any reduction in L.E. activity of the supernatant sera after incubating L.E. serum with one-day-old intact leukocytes serves to exclude the possibility that lupus factor could be adsorbed by the cytoplasm.

Haserick et al. found that L.E. factor was present only in the gamma globulin fraction of serum. He further believes that L.E. factor is immunologically distinct from other gamma globulins.

In this connection it should be stressed that systemic lupus erythematosus is characterized by a host of serologic disturbances which suggest profound alterations in protein metabolism. The presence of hypergammaglobulinemia, positive cephalin flocculation, false-positive serologic reactions for syphilis, auto-immune hemolytic anemia, and circulating anticoagulants, in addition to the L.E. cell phenomenon, bear witness to this. The in vivo passive transfer of L.E. factor to the offspring of lupus mothers is reminiscent of the placental transfer of antibodies. We recently observed the simultaneous placental transfer of L.E. factor and platelet antibodies; both the blood of mother and newborn demonstrated L.E. cell formation, thrombocytopenia and in vitro antiplatelet activity. The well known, favorable effects of corticosteroids, not only on the general condition, but also on many of the serologic disturbances in some patients with systemic lupus erythematosus, bears a striking resemblance to a similar therapeutic response in other well established immunologic disorders. Further studies are needed and are in progress to explore the immunologic nature of this disease.

How can the results of these experiments throw light on the mechanism of the L.E. cell test as it is performed in the laboratory? At the present time the formation of L.E. cells remains the only method available to demonstrate the presence of L.E. factor. It is assumed that the intensity of L.E. cell formation reflects the level of the circulating factor. The possibility exists, however, that just as there is no correlation between the severity of the clinical state and the intensity of L.E. cell formation in many patients with systemic lupus erythematosus, presently available morphologic tests may not give a quantitative appreciation of the level of the circulating L.E. factor. The present studies, while they clearly point up the specific affinity of bare leukocyte nuclei for L.E. factor, at the same time appear to elucidate the relationship of the living leukocyte to the overall process. There is much to suggest that the role of the viable leukocyte in the process of the formation of L.E. cells is not simply one of phagocytosis. The adsorption of L.E. factor to isolated nuclei does not result in the morphologic changes of swelling, homogeneity
and staining characteristic of the typical lupus body. The presence of viable leukocytes is in some way essential for the characteristic transformation of this material frequently seen as large extracellular masses (globs).

Another point also requires a special discussion. L.E. cell formation is initiated by the union of L.E. factor to cell nuclei, but at the same time an intact cytoplasm acts as a barrier to this union. Thus, the formation of L.E. cells, often in large numbers within 15 minutes in freshly clotted blood drawn from a lupus patient, requires explanation. The increase in the number of L.E. cells with the extension of the incubation time has been repeatedly observed. We have ascribed this to the progressive increase in the number of nonviable cells available for L.E. cell transformation. But, as we have shown in this study, intact leukocytes after considerable aging fail to adsorb L.E. factor. It appears likely, therefore, that in preparations using intact dead cells, the presence of viable cells is in some way essential to promote the union of L.E. factor to the cell nuclei of the former. Injured or dead cells may be attacked by the remaining viable leukocytes in such a way that the integrity of the cytoplasmic envelope is lost. The nuclei of these cells then can unite with L.E. factor and under the influence of living leukocytes become transformed into L.E. cell material, the so-called “globs,” “hematoxylin” or lupus bodies. The “globs” which abound in positive L.E. cell preparations are then phagocytized, and the resulting cell stuffed with one or more inclusions is a typical Hargraves' cell.

**Summary**

1. Isolated leukocyte nuclei quantitatively adsorb lupus factor from serum. In the presence of viable leukocytes such nuclei which have adsorbed the lupus factor become transformed into typical L.E. cells.
2. Leukocytes with an intact cytoplasm fail to adsorb L.E. factor.
3. These studies suggest that the living leukocytes, apart from simple phagocytosis, serve other functions in the L.E. phenomenon:
   (a) the disruption of the integrity of the cytoplasmic envelope of dead leukocytes so as to allow for the adsorption of L.E. factor to the cell nucleus.
   (b) the transformation of the nuclei, to which L.E. factor has been adsorbed, into swollen homogenous lupus bodies.

**Summario in Interlingua**

1. Isolate nucleos leucocytic adsorbe factor de lupus ab sero. In le presentia de viabile leucocytes tal nucleos, post que illos ha adsorbite le factor de lupus, deveni typic cellulas “L.E."
2. Leucocytos con cytoplasma intacte non adsorbe factor “L.E.”
3. Iste studios suggere que le leucocytos vive, a parte le simple phagocytosis, servi altere functiones in le phenomeno de “L.E.” Istos es:
   (a) le disruption del integrite del inveloppe cytoplasmic del leucocytos morte con le resultato que le nucleos cellular pote adsorber le factor “L.E.” e
   (b) le transformation del nucleos que ha jam adsorbite factor “L.E.” in inflate e homogenee corpores de lupus.
INTERACTION IN THE L.E. CELL PHENOMENON

ADDENDUM

Since the preparation of this manuscript, Holman and Kunkel (Science 126: 3265, 1957) reported on the adsorption of L.E. factor by the nuclei of human monocytes, rabbit leukocytes, and calf thymocytes. They further demonstrated that nucleoprotein released from the nuclei also adsorbed L.E. factor and became transformed into L.E. cells when incubated with living white cells.

REFERENCES

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