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MORPHOLOGICAL STUDIES ON ALLERGEN INDUCED BLAST TRANSFORMATION OF SENSITIZED LYMPHOCYTES

By

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The blast transformation of lymphocytes, either non-specific (phytohemagglutinin induced) or specific (due to the action of various allergens), is a biological phenomenon which has recently aroused deep interest. The blast transformation test of lymphocytes has proved to be one of the most reliable methods at present for the detection of immediate and delayed type allergic reactions (4, 7, 8, 11, 12). Our studies along these lines showed that the test may be efficiently applied to the diagnosis of grass pollen and penicillin allergy. When compared to other *in vitro* methods, such as passive hemagglutination, latex agglutination, or basophil degranulation, it proved to be superior (i.e. better reproducibility and specificity) (9, 10).

The utilization of the blast transformation test in the diagnosis of allergic states poses some problems as to: the role of the T and B lymphocytes in this phenomenon, relevant to the capacity of T and B lymphocytes to undergo blast transformation; the role of the antigen in the stimulation of one or the other cell type; and hence the nature of the subsequent blast transformation (1, 2, 3, 5). Thus, a good knowledge of the ultrastructure of the blast cells becomes indispensable. In this connection, the morphological and especially the ultrastructural changes occurring in the lymphoblast cells, evoked by grass pollen and penicillin were investigated.

MATERIALS AND METHODS

Allergens

Grass pollen allergen (a product of the allergy laboratory of the Center for Infectious and Parasitic Diseases) in concentration of 10000 PNU/ml and penicillin 30000 U/ml were employed.

Lymphocyte Transformation Test

1. In order to obtain pure lymphocytes, 10 ml peripheral blood was drawn from the cubital vein of allergic patients (some of them sensitized to grass pollen and the others to penicillin), who manifested clear-cut anamnestic and clinical symptoms. The blood was processed by the two-step centrifugation procedure of Kelley (8), as described previously (9, 10). The resulting lymphocytes showed 95 per cent purity and were viable.

2. Cultivation of the lymphocytes took place in Eagle's medium supplemented with 20 per cent homologous serum and the corresponding allergen—10000 PNU/ml grass pollen and penicillin 30000 U/ml at 37° C for 96 hours. Details about this procedure have been published elsewhere (9, 10).

3. After the cultivation was finished, microscopic preparations of the cell suspensions were stained according to Romanovski-Giemsa and examined under the light microscope. The remaining cells were subjected to electron microscopy by the method of Ilkov et al. (6). An electron microscope SEM 3 (GDR) at 8000 to 12000 $\times$ magnification was used.

RESULTS AND DISCUSSION

Previous studies (9, 10) revealed certain differences in the nature of the blast cells, probably due to the stimulation of the sensitized lymphocytes by various antigens/allergens. The present data also indicate the presence of different blast cell types, as demonstrated by using pollen and penicillin as stimulators. The blast cells obtained after pollen stimulation appeared to be larger in size (15 to 20 μ m), they had strongly vacuolized foamy protoplasm; and the nucleus:protoplasm ratio was shifted a considerable distance towards the protoplasm. The penicillin induced blast cells were smaller (12 to 15 μ m); the nuclei manifested enhanced optical density; the vacuolization of the protoplasm was not marked; and the nucleus:protoplasm ratio was not so great. These differences, detected by light microscopy, are only tentative, and that prompted further electron microscopic studies. The latter

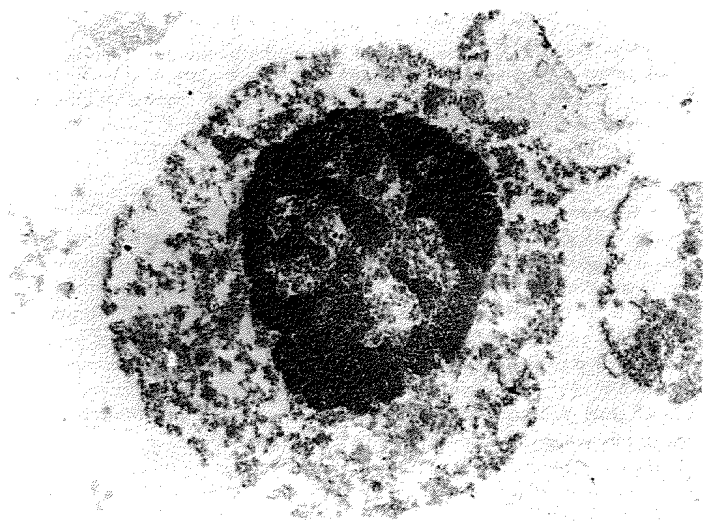


Fig. 1.

Blast cell from a lymphocyte culture stimulated by grass pollen allergen. Strongly enhanced nucleus:protoplasm ratio. In the protoplasm there are a great number of mitochondria, well-developed Golgi apparatus and many free ribosomes. 8000 $\times$.

disclosed a large number of destroyed blast cells with disrupted cell walls, especially in lymphocyte cultures derived from patients sensitized to grass pollen allergen (Fig. 1). It is likely that these findings may be due to the long treatment of the cells prior to durcupan embedding. What is of major importance is that electron microscopic examination failed to reveal significant morphological differences between the blast cells in both groups. An enhanced number of free ribosomes in the cytoplasm, mitochondria and well-developed Golgi apparatus were visible (Fig. 2). These findings, along with the usually observed increased accumulation of ribosomes in the cytoplasm, provide conclusive evidence of the blast transformation of lymphoid cells (Fig. 3).

As mentioned already, our interest in the morphology of

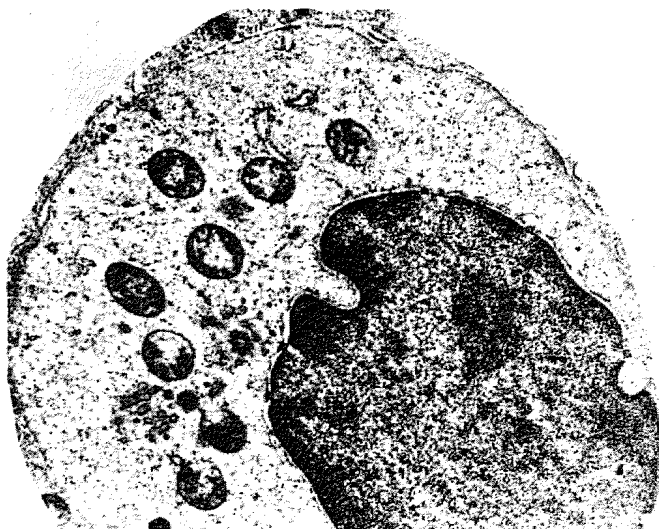


Fig. 2.

Blast cell from a lymphocyte culture stimulated by penicillin. In the protoplasm there are a great number of polysomes and well-developed Golgi apparatus. 8000 X.

the blast cells stemmed from the fact that numerous controversial data on the role of T and B lymphocytes have been obtained. It is obvious that the older concept of the predominant role of the T cells in the blast transformation should be challenged, since the mechanism of action of the atopic allergen grass pollen (capable of inducing typical blast transformation) in the human organism is associated with B-cells and the subsequent production of IgE antibodies. At present two explanations can be offered. It is possible that the weak pollen allergen stimulates the T-lymphocytes, which have a lower threshold of sensitivity than B-lymphocytes, as suggested by Mitchison (3). There are other authors however (1) who assume that the pollen acts directly on B-lymphocytes and their subsequent transformation, but this is at variance

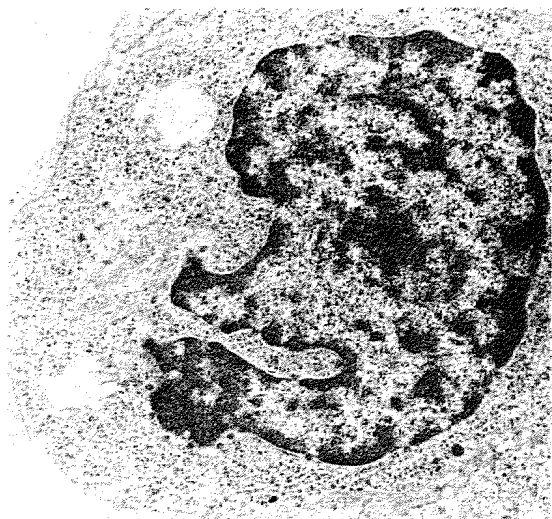


Fig. 3.

Blast cell from a lymphocyte culture stimulated by grass pollen allergen. Strongly enhanced nucleus:protoplasm ratio. In the protoplasm there are a great number of mitochondria, well-developed Golgi apparatus and many free ribosomes. 8000 $\times$.

with existing views on the role of T and B lymphocytes in the immunological reactivity of the organism. Moreover, in conjunction with blast transformation and other immunological reactions in the organism, such as antibody formation, it may be assumed that one single lymphocyte type comprising cells in different physiological states may present different functional patterns, depending on altering conditions.

In order to furnish some information on this aspect, we attempted the blast transformation of the lymphocytes by grass pollen allergen (a typical B-dependent antigen) and by penicillin. As already stated, we did not establish any significant differences in the ultra-structure of the blast cells induced by either allergen. Some authors claim to have observed some characteristic differences in the ultrastructure of T and B

lymphocytes at the electron microscopic level, allowing a differentiation between these cell types, and to relate them to certain biological phenomena occurring in the organism. Such observations were not confirmed in the present study, and not all studies agree on this point. A straightforward answer to this may be expected in the near future.

SUMMARY

The morphological and ultrastructural changes occurring in blast transformed lymphocytes, isolated from patients sensitive to grass pollen and penicillin, were studied. Certain differences in the appearance of the blast cells, as evidenced by light microscopic observation, were not found by electron microscopy. The blast cells showed foamy protoplasm regardless of the nature of the allergens employed. An increased number of either single or rosette-forming ribosomes was established. Numerous vesicles were visible in the cytoplasm. The endoplasmic sacs were more numerous. Golgi apparatus was well-developed; the mitochondria were increased and had a dense matrix.

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