DIFFERENCES IN THE PROPERTIES OF LYMPHOCYTES IN HODGKIN'S DISEASE AND CHRONIC LYMPHATIC LEUKEMIA

T. K. Veskova, I. L. Duk, and G. Ya. Svet-Moldavskii

UDC 616-006.442+616.155.392.2-036.12]-07: 616.155.32-07

Depending on the intensity of their adhesion to cells of a Hep-2 monolayer, the circulating blood lymphocytes of patients can be arranged in the following order: chronic lymphatic leukemia > normal > Hodgkin's disease. Depending on the intensity of formation of DNA-containing "bridges" or projections, the lymphocytes are arranged in the opposite order: Hodgkin's disease > normal > chronic lymphatic leukemia.

The properties of morphologically similar lymphocytes can vary considerably in different diseases. Tests to determined these properties are only beginning to be developed.

A characteristic feature of Hodgkin's disease is the suppression of immunologic reactions of the delayed type and of transplantation immunity, together with only slight disturbance of antibody production [6, 10]. Chronic lymphatic leukemia is characterized to a definite extent by the opposite situation: marked depression of antibody formation but a relatively less marked disturbance of immunologic reactions of the delayed type [1, 13]. Both in Hodgkin's disease and in chronic lymphatic leukemia (CLL) the lymphocytes are transformed with difficulty into blast cells [2, 6, 8, 11, 12, 14].

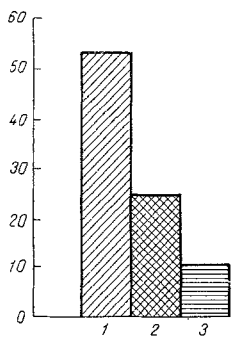

Fig. 1. Adhesion of lymphocytes to Hep-2 target cells after 12 h: 1) lymphocytes of patient with lymphatic leukemia; 2) of healthy donor; 3) of patient with Hodgkin's disease.

In both diseases the lymphocytes produce little interferon during contact with virus interferonogen [3]. Finally, in the presence of phytohemagglutinin (PHA) the lymphocytes of patients with lymphogranulomatosis and of patients with CLL have a much weaker cytotoxic action on allogeneic and heterogeneic target cells than the lymphocytes of healthy persons [9, 15].

In the investigation described below the behavior of lymphocytes of patients with the two diseases toward target cells and in tissue culture was studied.

EXPERIMENTAL METHOD

Heparinized blood from healthy donors and patients with Hodgkin's disease and CLL was treated with 10% gelatin in the proportion of 1 ml gelatin solution to 10 ml blood, and the mixture was kept at 37°C for 30-40 min to allow the erythrocytes to settle. The top layer of plasma with leukocytes was drawn off. Neutrophils were removed from the suspension of leukocytes by the method of Bach and Hirschhorn [7], based on the increased tendency of neutrophils to adhere to the flask wall. The cell suspension then contained from 70 to 98% of lymphocytes.

Laboratory of Virology, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR E. M. Tareev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 1, pp. 49-51, January, 1971. Original article submitted June 26, 1970.

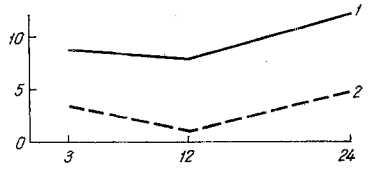

Fig. 2

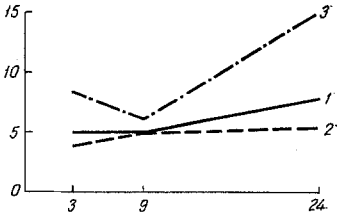

Fig. 3

Fig. 2. Formation of DNA-containing bridges by lymphocytes adherent to Hep-2 target cells in the presence of PHA. Here and in Fig. 3: abscissa, time in h; ordinate, percentage of lymphocytes forming bridges. 1) Lymphocytes of normal donor; 2) lymphocytes of patient with chronic lymphatic leukemia.

Fig. 3. Formation of bridges by lymphocytes adherent to Hep-2 target cells: 1) lymphocytes of normal donor; 2) lymphocytes of patient with chronic lymphatic leukemia; 3) lymphocytes of patient with Hodgkin's disease.

Transplantable human Hep-2 cells were used as target cells. Cover slips were placed on the bottom of insulin flasks. Each flask was then seeded with Hep-2 cells at the rate of 50,000 per ml of medium No. 199 +10% bovine serum. Usually a thin monolayer formed in the cover slip in 24 h. In some experiments 1.25-2.5 μl/ml of phytohemagglutinin (Difco) was added to the system. The nutrient medium with bovine serum was then withdrawn from the flasks and replaced by 1 ml of a suspension of lymphocytes per flask, made up to a concentration of 500,000 or 1,000,000 cells per ml of medium No. 199. The flasks were incubated at 37°C.

After various time intervals the slips with the monolayer of Hep-2 cells and adherent lymphocytes were fixed. The medium was drawn off and the cover slip with the culture was rinsed carefully twice with medium No. 199 and fixed for 5 min with Carnoy's mixture. The specimens were stained with azure-eosin and by Feulgen's method. Under the microscope, 200 cells with adherent lymphocytes were counted. The number of lymphocytes was then expressed per 100 Hep-2 cells. The DNA-containing projections or bridges between the lymphocytes and Hep-2 cells, described by Sura et al., [4], were then counted on 200 lymphocytes, and the result expressed per 100 lymphocytes. Projections or bands outwardly appearing to connect the lymphocyte to the target cell were counted as bridges. A parallel study of adhesion and of the formation of DNA-containing bridges was made in specimens with lymphocytes from the blood of a normal donor and of a patient with CLL or with Hodgkin's disease. The numerical results were conventionally regarded as different if they differed by as much as 50%. Otherwise they were taken as equal.

EXPERIMENTAL RESULTS

Although the absolute numerical indices of adhesion and the formation of DNA-containing bridges varied in different experiments, in all eight experiments the lymphocytes of the patients with CLL adhered more strongly than the lymphocytes of patients with Hodgkin's disease: sometimes by many times, sometimes by only 1.5 times (Fig. 1). In eight of the 11 experiments the lymphocytes of patients with CLL adhered more strongly to the Hep-2 cells than normal lymphocytes, while in the other three they adhered equally. Conversely, the lymphocytes of patients with Hodgkin's disease adhered less strongly than normal in four of the eight experiments, in three as strongly as normal, and in one experiment more strongly than normal. The impression was obtained that by their degree of adhesion the lymphocytes could be arranged in the following order: CLL > normal > Hodgkin's disease. It is important to note that on the addition of PHA, which sharply stimulated adhesion, this difference remained. The difference between the lymphocytes of patients with CLL and with normal lymphocytes, however, became more marked after the addition of PHA.

The opposite relationships were observed with the DNA-containing bridges from lymphocytes to Hep-2 cells. Their formation in the system with lymphocytes of patients with CLL occurred in nine of the 10 experiments to a lesser degree than in the system with normal lymphocytes, and in one experiment to the same degree. Conversely, in eight of the 12 experiments the lymphocytes in Hodgkin's disease formed

more bridges than the lymphocytes of patients with CLL, even though at only one time of fixation. In eight of the 10 experiments the lymphocytes of patients with Hodgkin's disease formed these bridges more intensively than normal lymphocytes or equally so. In other words, the formation of DNA-containing projections (bridges) showed the following tendency: Hodgkin's disease > normal > chronic lymphatic leukemia (Figs. 2 and 3).

In 1969 Thomson and Menrishi [16] found a higher degree of adhesibility of the lymphocytes in CLL to polystyrene beads, especially in the presence of the serum of patients with this disease, and evidently due to differences in the surface properties of these lymphocytes.

Adhesion of lymphocytes to cells of an Hep-2 monolayer is a complex phenomenon. This method takes into account only lymphocytes which adhere strongly enough to withstand rinsing of the monolayer. The whole process, leading to one picture or the other, is composed of sedimentation of the lymphocytes on the monolayer, the actual adhesion, and subsequent interactions between lymphocytes and cells of the monolayer. The strength of contact between lymphocytes and cells of the monolayer probably depends on many factors, but primarily on the surface properties of the lymphocytes.

In fact, the adhesibility of the lymphocytes of patients with CLL is evidently higher still, since with the method used to remove granulocytes, it is probable that some of the more strongly adherent lymphocytes also were lost.

DNA-containing projections or bridges, crossing from the nuclei of the lymphocytes to the target cells, were described in 1967 by Sura et al. [4]. Subsequent electron-microscopy studies [5] showed that at least some of these bridges are fragments of the disintegrating lymphocyte nucleus undergoing phagocytosis by the cytoplasm of the target cell. The role of these bridges is unknown, but it is evident that their ability to be formed in the lymphocytes in Hodgkin's disease differs greatly from that in CLL. The appearance of a large number of these structures perhaps indicates the greater vulnerability of the lymphocytes in Hodgkin's disease.

LITERATURE CITED

1. É. G. Bragina, M. A. Volkova, L. D. Grinshpun, et al., in: Problems in Leukemia. Epidemiology and Epizootology. Proceedings of a Symposium [in Russian], Riga (1968), p. 145.
2. N. I. Braude, L. D. Grinshpun, and M. I. Kryuchkov, Vestn. Akad. Med. Nauk SSSR, No. 4, 65 (1968).
3. B. M. Nemirovskaya and T. K. Veskova, Vopr. Onkol., No. 4, 52 (1970).
4. S. N. Sura, I. Yu. Chernyakhovskaya, Z. G. Kadagidze, et al., Byull. Éksperim. Biol. i Med., No. 11, 103 (1967).
5. S. N. Sura, A. F. Bykovskii, Z. G. Kadagidze, et al., Byull. Éksper. Biol. i Med., No. 9, 98 (1970).
6. A. C. Aisenberg, Cancer Res., 26, 1152 (1956).
7. F. Bach and K. Hirschhorn, Science, 143, 813 (1964).
8. M. W. Chase, Cancer Res., 26, 1097 (1966).
9. G. Holm, P. Perlmann, and B. Johanneson, Clin. Exp. Immunol., 2, 351 (1967).
10. D. G. Miller and J. G. Lizardo, J. Nat. Cancer Inst. (Washington), 26, 569 (1961).
11. J. J. Oppenheim and J. Whang, Blood, 26, 121 (1965).
12. A. D. Rubin, K. Havemann, and W. Dameshek, Blood, 33, 313 (1969).
13. R. K. Shaw, C. Szwed, D. R. Boggs, et al., Arch. Intern. Med., 106, 467 (1960).
14. J. W. Thomas, W. Boldt, G. Horrocks, et al., Canad. Med. Assn. J., 97, 832 (1967).
15. J. W. Thomas, W. Boldt, and G. Horrocks, Canad. Med. Assn. J., 99, 303 (1968).
16. A. S. R. Thomson and J. N. Menrishi, Europ. J. Cancer, 5, 195 (1967).