RESPONSE OF LYMPHOCYTES OF PATIENTS WITH SCHIZOPHRENIA TO PHYTOMITOGENS CONCANAVALIN A AND PHYTOHEMAGGLUTININ

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The ability of lymphocytes of patients with schizophrenia and of healthy subjects to respond to the stimulant action of T mitogens (concanavalin A and phytohemagglutinin) was studied: The number of T cells was determined by the rosette-formation method and the effect of adhesive cells on the response of the lymphocytes to the mitogens was investigated. The response to both mitogens was depressed in cultures of the patients' lymphocytes compared with the control, but the number of T cells was indistinguishable from normal. Removal of the adhesive lymphocytes leads to disappearance of the differences between the responses of the patients' and healthy subjects' lymphocytes to both mitogens.

KEY WORDS: schizophrenia; lymphocyte; phytohemagglutinin; concanavalin A.

The study of the peripheral blood lymphocytes of patients with schizophrenia has revealed certain special features of the physiological state of these cells. Blood of these patients has been shown [1, 5] to contain a subpopulation of lymphocytes distinguished by their active physiological state and their marked adhesive properties (atypical lymphocytes). Meanwhile, in experiments to assess the ability of lymphocytes to respond to the stimulating action of mitogens, a smaller proportion of the lymphocytes of schizophrenic patients than of normal subjects was found to respond to phytohemagglutinin (PHA), a T mitogen [3]. It was accordingly concluded that in schizophrenic patients there is a subpopulation of functionally altered, possibly defective, T lymphocytes, or an actual deficiency of T cells in the peripheral blood of these patients. However, more recent observations have shown that not all T lymphocytes can respond to any one T mitogen [12, 13] and that at least two T mitogens must be used for the objective assessment of the state of all the T cells in the lymphocyte population. Such more complete assessment is very important for the elucidation of the role of T lymphocytes in the change in the reactions of cellular immunity in schizophrenia.

It was accordingly decided to study the response of the peripheral blood lymphocytes of patients with schizophrenia to two T mitogens: PHA and concanavalin A (con A). An attempt also was made to assess the role of adhesive activated lymphocytes in the response of the whole population to these T mitogens.

EXPERIMENTAL METHOD

A group of 12 patients with schizophrenia and a control group of 17 healthy blood donors were studied. The schizophrenic group consisted of four patients with a continuous-progressive form, seven with an episodic-progressive form, and one patient with a periodic form of schizophrenia. The whole lymphocyte population was obtained together with all the white blood cells after sedimentation of the erythrocytes in tubes from the peripheral blood after the addition of heparin (13-15 i.u./ml). The fraction of nonadhesive lymphocytes also was obtained after passing the leukocytes through a column containing glass beads [11]. The purity of the resulting fraction was over 90%. The number of cells in the suspension obtained by the methods described above was counted and the suspension was diluted to a concentration of 1 million cells to 1 ml of medium containing 20% autologous serum and 80% Eagle's medium with glutamine, after which 1 ml of the suspension was poured

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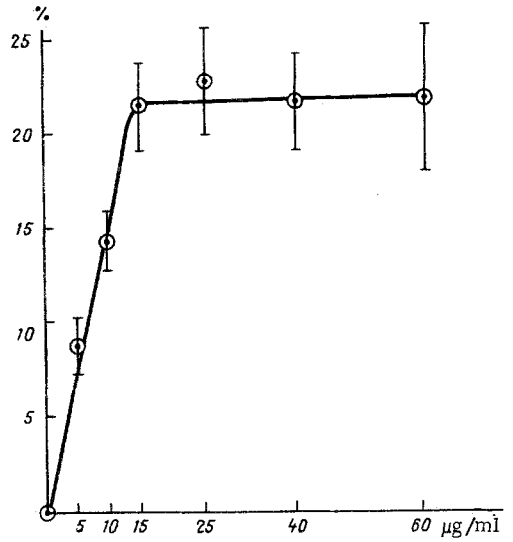

Fig. 1

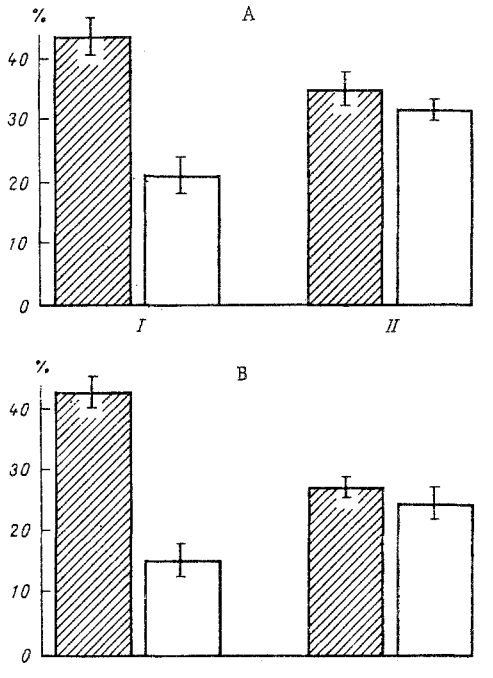

Fig. 2

Fig. 1. Percentage of lymphocytes with increased DNA content in cultures stimulated by different concentrations of con A. Abscissa, concentration of con A (μg/ml); ordinate, percentage of responding cells.

Fig. 2. Response of lymphocytes to stimulation by PHA (A) and con A (B). Unshaded columns represent patients, shaded columns healthy subjects. I) Initial population of lymphocytes; II) subpopulation of nonadhesive lymphocytes. Ordinate, percentage of lymphocytes with increased DNA content.

into each of a series of penicillin flasks for incubation at 37°C. PHA (Difco, USA) and con A (Sigma, USA) were added to the corresponding flasks up to a final concentration of 40 and 20 μg/ml, respectively. The cells were collected and fixed after 1 and 72 h in culture. To obtain each preparation the contents of at least two flasks with parallel cultures were used. The reaction of the lymphocytes to the mitogens was assessed by counting cells with an increased DNA content. To determine the DNA content all preparations were stained simultaneously by Feulgen's method [4], after which coverslips with the stained cells were dried in air and mounted in Canada balsam. The DNA content was determined with a scanning cytophotometer (Opton, West Germany) at a wavelength of 570 nm. At least 50 cells were measured in each preparation. Lymphocytes with a DNA content exceeding the mean DNA content in the cells of 1-h cultures by more than 3σ, were classed as cells responding to stimulation. In some experiments the relative percentage of T lymphocytes was determined at the same time by the spontaneous rosette-formation method [7].

EXPERIMENTAL RESULTS

In this investigation concanavalin A (con A) was used for the first time to study the functional state of the lymphocytes in schizophrenia. Several investigations of lymphocytes from normal subjects and in patients with various diseases in which con A has been used as a mitogen have been described in the literature [8-10], but because of the contradictory nature of the data given by different workers it was not clear what concentration of mitogen should be used in the experiments with cells from schizophrenic patients; for that reason the action of various concentrations of con A on the lymphocytes of these patients was studied. The results are given in Fig. 1, from which it follows that when con A was used in concentrations of 15 to 60 μg/ml the percentage of transformed cells was unchanged. Accordingly, in the subsequent experiments con A was used in a concentration of 20 μg/ml.

The study of the response of the peripheral blood lymphocytes of schizophrenic patients to stimulation by T mitogens showed that the percentage of responding cells in cultures of lymphocytes with PHA was 20.2 ± 3.1,

and in cultures with con A 14.9 ± 2.5. In control cultures of healthy human lymphocytes the percentages of these cells were 43.6 ± 2.9 and 42.0 ± 2.7, respectively. These results are shown graphically in Fig. 2, from which it is clear that the response of the lymphocytes of the schizophrenic patients to both T mitogens was substantially less than normal. In the case of PHA the difference in the response was 23.4% ($P < 0.001$), whereas in the case of con A it was 27.1% ($P < 0.001$). Preliminary analysis of the data reflecting the response of lymphocytes of patients with different forms of schizophrenia to con A showed that the response of lymphocytes obtained from patients with an episodic-progressive type of course was substantially weaker than the response of cells from patients with a continuous-progressive form of schizophrenia.

It is also clear from Fig. 2 that in cultures of cells both from patients and from healthy subjects approximately equal numbers of lymphocytes responded to both mitogens. This could be explained by the fact that the same subpopulation of T lymphocytes responds to both PHA and con A. In that case, however, correlation ought to be observed between the responses of the lymphocytes to PHA and to con A. No such correlation was found (in the cultures of patients' cells $r = 0.27$, in cultures of healthy lymphocytes $r = 0.38$). Consequently, different subpopulations of T lymphocytes most probably respond to PHA and to con A. This view is supported by the observations of other workers who have specially studied this problem [13]. Meanwhile there is another point of view, based on experimental data, namely that the whole population of T lymphocytes, including PHA-reacting cells, responds to con A [12]. It is evident that in that case also direct correlation ought to be observed between the percentage of lymphocytes responding to con A and to PHA. The data described above thus make it appear more likely that the hypothesis that basically different cells respond to con A and to PHA is correct, although evidently there are some lymphocytes which can respond to both mitogens.

Evidence of the diminished response of lymphocytes of schizophrenic patients to con A and to PHA was given above. This effect could be due either to a deficiency of T cells or to their defective function in schizophrenia. There is evidence in the literature that no deficiency in the number of T lymphocytes is observed in the blood of schizophrenic patients [2]. The results of the present investigation agree with this view. In experiments to determine the percentage of lymphocytes spontaneously forming rosettes with sheep's red cells (T lymphocytes) their number in the blood of the schizophrenic patients was 55.8 ± 9.1% compared with 56.8 ± 3.4% of T lymphocytes in the blood of the healthy donors. These figures agree with many others cited for the number of T cells in healthy human blood [2, 6]. The hypothesis of defective function of a certain proportion of the T cells in schizophrenia thus seems to be the most likely one.

As was already mentioned, "atypical" lymphocytes are found in the blood of patients with schizophrenia; these lymphocytes are distinguished by their marked adhesive properties and their more active physiological state, and it was therefore interesting to discover the role of these cells in the diminished response of the lymphocytes to T mitogens in schizophrenia. To study this problem the ability of the atypical activated lymphocytes to adhere to the surface of glass was used, for these cells can be removed from the general population by the use of columns with glass beads [5]. It was expected that if these atypical cells are in fact responsible for the difference between the schizophrenic and healthy lymphocytes, the response of the cells in the nonadhesive fraction of lymphocytes from schizophrenic patients would be the same as the response of the corresponding fraction of healthy blood. The results of these experiments are given in Fig. 2. Clearly, after removal of the adhesive cells the percentage of the patients' lymphocytes responding to PHA and to con A (32.2 ± 1.4 and 24.7 ± 2.6, respectively) was equal to the percentage of responding nonadhesive healthy lymphocytes (35.6 ± 2.6 and 27.5 ± 1.4, respectively). Hence it follows that the weaker than normal response of the lymphocytes in cultures of peripheral blood cells of schizophrenic patients is due to the presence of adhesive activated lymphocytes, which evidently respond neither to PHA nor to con A.

It is interesting to note that removal of adhesive lymphocytes leads to completely opposite changes in the responses of the patients' and donors' lymphocytes: In cultures of lymphocytes from patients with schizophrenia the response to both mitogens was increased, whereas in cultures of healthy donors' lymphocytes it was reduced (Fig. 2). These facts can be explained on the assumption that the adhesive lymphocytes of patients with schizophrenia are in a different functional state from that of donors' adhesive lymphocytes.

These experiments thus showed that the peripheral blood of patients with schizophrenia contains the normal number of T lymphocytes; however, by contrast with normal subjects, a certain proportion of these cells does not respond to the stimulating action of the T mitogens con A and PHA.

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EFFECT OF AVITAMINOSIS B_6 IN MICE ON T-LYMPHOCYTE FUNCTION in vitro

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The effect of different degrees of avitaminosis B_6 in mice on the cytolytic activity of T lymphocytes, measured as the quantity of $Na_2Cr^{51}O_4$ released from lysed target cells, was studied on a model of the primary immune response in a mixed lymphocyte culture in vitro. Keeping animals for 3 weeks on a diet without pyridoxine did not affect the ability of the lymphocytes to proliferate in vitro or their cytolytic activity. In animals receiving a diet without pyridoxine for 45 days the content of pyridoxal-5'-phosphate in the spleen was 55% lower than in the control. Lymphocytes taken from these animals, when cultured in vitro, showed sharply weakened ability to incorporate [^{3}H]thymidine into DNA in response to the alloantigen. The cytolytic activity of these lymphocytes also was reduced. The ability of different forms of pyridoxine to restore the functions of T lymphocytes, when disturbed by avitaminosis B_6, was studied.

KEY WORDS: avitaminosis B_6; pyridoxine; pyridoxal; pyridoxal-5'-phosphate; cytolytic activity.

Mammalian lymphoid tissue is extremely sensitive to a deficiency of pyridoxine in the diet [7, 11]. Avitaminosis B_6, induced in experimental animals by restriction of the pyridoxine intake with the food or by injection of antagonists of vitamin B_6 causes a disturbance of both humoral [5, 8] and cellular [4, 10] immunity. Robson and Schwarz [9] showed recently that, during in vitro culture of lymphocytes taken from rats kept for 2 weeks on a diet deficient in vitamin B_6 the incorporation of [^{3}H]uridine into the DNA of the cells in response to an alloantigen is sharply depressed.

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