ROLE OF ALLERGIC REACTIONS OF PERIPHERAL BLOOD LEUKOCYTES IN THE PATHOGENESIS OF EXPERIMENTAL ALLERGIC (PERTUSSIS) ENCEPHALOMYELITIS

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After sensitization of guinea pigs with an oily suspension of *Bordetella pertussis* cells, starting from the 14th day a persistent leukocytosis and lymphocytosis was observed in the peripheral blood of the animals. The leukocytic formula showed a shift to the left. On contact between the blood leukocytes and specific brain antigen both *in vivo* and *in vitro* the leukocytolysis and allergic changes in the leukocytes observed in the late stages of sensitization (in the period of clinical manifestations of experimental allergic encephalomyelitis — EAE) took place mainly at the expense of the granulocytes. These changes are tentatively attributed to anti-brain antibodies present in the blood in this period of EAE, i.e., to a combination of allergic reactions of immediate type and reactions of delayed type in a common mechanism of development of pertussis EAE.

KEY WORDS: *experimental allergic encephalomyelitis; allergic changes in the leukocytes.*

By the use of experimental allergic encephalomyelitis (EAE) as a model [5] to study the reactivity of peripheral blood lymphocytes toward brain antigen *in vitro* [3] it has been shown that in the late stages of sensitization in the period of appearance of the clinical picture of pertussis EAE the reactivity of the lymphocytes was depressed. The possible causes of this phenomenon may be either elimination of sensitized lymphocytes from the blood stream or a decrease in the total number of circulating lymphocytes.

In this investigation, in order to answer this question and also to study the reactivity of granulocytes toward brain antigen *in vivo* and *in vitro*, an investigation was made of the total number of leukocytes and the leukocytic formula at various stages of sensitization of animals, of leukocytolysis *in vivo*, and of allergic changes in the leukocytes *in vitro*.

EXPERIMENTAL METHOD

Guinea pigs weighing 250-300 g of both sexes were used. Pertussis EAE was produced by the method described by the writers earlier [3]. Saline extracts of bovine brain, liver, and kidney prepared by the method of David and Paterson [7] were used as antigens for the reactions *in vivo* and *in vitro*. A group of intact guinea pigs served as the control.

At various times after sensitization blood was taken from the marginal vein of the ear of the animals in order to determine the total leukocyte count and formula. Specific brain antigen was then injected into the heart of these animals in a dose not sufficient to cause external manifestations of anaphylactic shock (group 1). As preliminary experiments showed, this dose was 0.5 ml of the 20% brain antigen: Its injection into sensitized animals did not cause a state of anaphylactic shock at any period of sensitization. The sensitized animals of group 2 received an injection of the same dose of nonspecific liver antigen. Blood was taken from the auricular vein of the animals 45-60 min after the injection and the total leukocyte count and formula were again determined.

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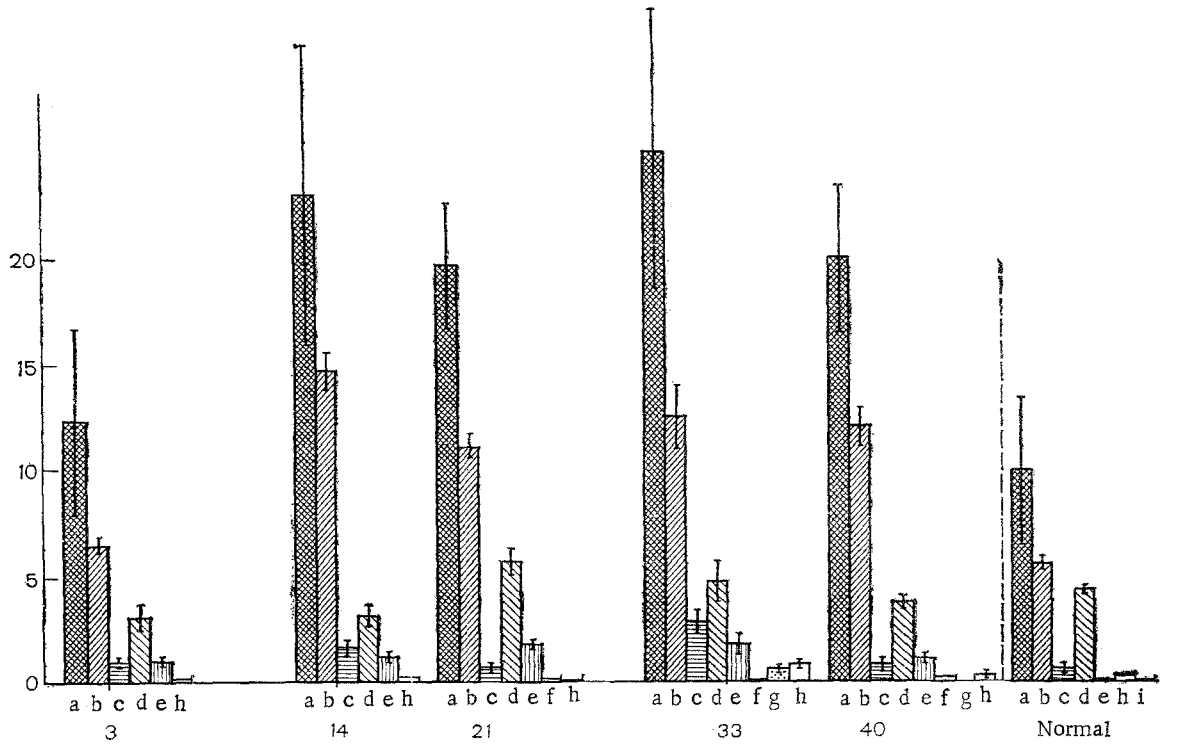

Fig. 1. Total leukocyte count and leukocyte formula of guinea pigs sensitized with oily emulsion of *B. pertussis* cells: a) total leukocyte count; b) lymphocyte; c) monocyte; d) polymorphonuclear neutrophils; e) stab neutrophils; f) young neutrophils; g) blast cells; h) eosinophils; i) basophils. Vertical lines show confidence limits for P = 0.05. Abscissa, days of sensitization; ordinate, number of cells (in thousands).

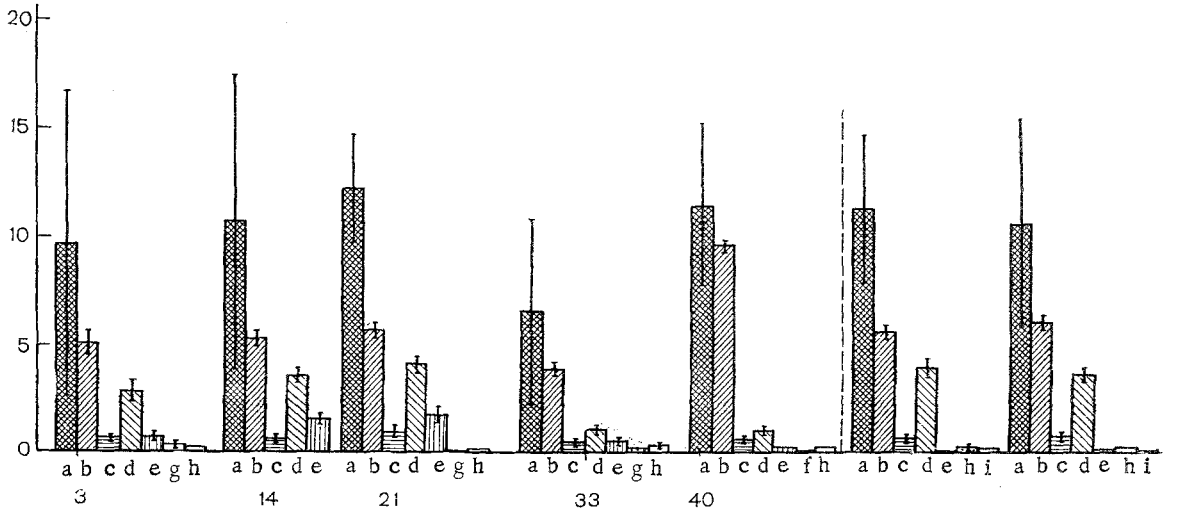

Fig. 2. Total leukocyte count and leukocyte formula of sensitized guinea pigs after injection of brain antigen into peripheral blood. Legend as in Fig. 1.

The total leukocyte count was determined by the standard method using a Goryaev chamber. The leukocyte formula was determined in blood films stained by the Romanovsky–Giemsa method, using the standard counting method. In order to evaluate the results, the indices of the leukocyte formula, expressed as percentages, were converted into absolute numbers.

The reactivity of the granulocytes toward brain antigen was demonstrated by the allergic alteration of granulocyte test *in vitro* in the direct modification [1]. For this purpose, blood was taken from the heart of the animals of group 3 at different times of sensitization, together with anticoagulant (a 1.5% solution of Chelaton) in the ratio of 1:2. This blood, in a volume of 0.45 ml, was incubated for 45-50 min in centrifuge tubes with 0.05 ml of brain antigen. The same volume of physiological saline and of antigen from bovine kidney was used

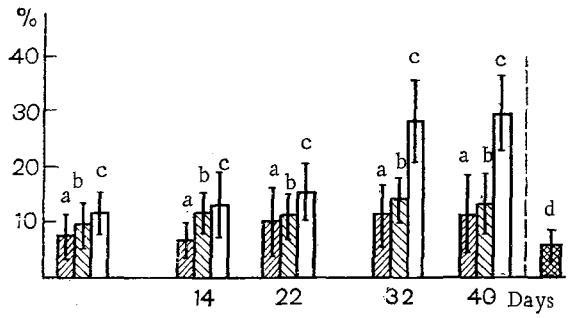

Fig. 3. Allergic alteration *in vitro* of granulocytes from guinea pigs sensitized with an oily emulsion of *B. pertussis* cells: a) without injection of antigen; b) kidney antigen; c) spinal cord antigen; d) normal granulocytes + spinal cord antigen. Vertical lines show confidence limits for P = 0.05.

as the control. The preparations were vitally stained with acridine orange (in a dilution of 1:20,000) and examined in the ML-2 luminescence microscope. Alteration of the granulocytes was expressed as a percentage by determining the number of altered cells in a differential count of 100 granulocytes.

EXPERIMENTAL RESULTS

Preliminary experiments were carried out on a group of 10 intact guinea pigs to determine the total leukocyte count and formula. The total number of leukocytes in 1 mm^3 blood was found to be 11,500 ± 1500 (here and later, M ± m). The leukocyte formula was as follows: basophils 0.1 ± 0.01% (114 ± 12), eosinophils 2.81 ± 0.7% (219 ± 84), neutrophils: young 0%, stab cells 0.3 ± 0.25 % (62 ± 57), polymorphonuclear 39.4 ± 2.6% (4581 ± 349), lymphocytes 50 ± 2.6% (5749 ± 287), and monocytes 6.1 ± 1.2% (757 ± 88). Comparison of these results with those obtained by other workers [2] showed no significant difference.

The total leukocyte count and the leukocyte formula were studied at various times of sensitization. The total leukocyte count increased starting from the 14th day of sensitization (23,210 ± 3100) and it remained high at all subsequent times of observation compared with the group of intact guinea pigs (Fig. 1). A regenerative shift of the leukocyte formula to the left was observed on the 3rd and 14th day of sensitization (Fig. 1), i.e., the number of stab cells was increased (1153 ± 173 and 1245 ± 167, respectively) and the number of polymorphonuclear neutrophils was reduced (3166 ± 260 and 3222 ± 424). Later, on the 21st day, the shift of the formula to the left became degenerative (stab cells 1821 ± 180, polymorphs 5841 ± 413) and it reverted to regenerative in character on the 33rd and 40th days of sensitization (stab cells 1988 ± 312 and 1252 ± 141, polymorphs 4833 ± 914 and 4387 ± 646, respectively).

Besides the changes in the leukocyte formula, starting with the 14th day and at all subsequent times of observation lymphocytosis was found, and on the 33rd day there was a monocytosis (3646 ± 834); marked eosinophilia also was present and blast forms appeared.

It is essential to note that in the late stages of sensitization so-called toxic granules appeared in the cytoplasm of the granulocytes; grosser forms of injury also appeared, in the form of vacuolation and degranulation of the cytoplasm and pycnosis of the nucleus.

To study the specific reactivity of the leukocytes *in vivo*, a dose of 20% saline extract of bovine spinal cord not sufficient to produce shock (0.5 ml) was injected into the heart. In control experiments injection of such a dose of spinal cord antigen (just as injection of nonspecific liver antigen) into intact guinea pigs (10) was not accompanied by any marked changed in the white blood picture. The total leukocyte count after injection of the spinal cord antigen was 10,800 ± 2100, and after injection of liver antigen 10,980 ± 1630. The relative percentage of eosinophils was 1.5 ± 0.5% (134 ± 27) and 2 ± 0.4% (218 ± 44), respectively, of basophils 0.5 ± 0.05% (58 ± 5) and 0.7 ± 0.1% (76 ± 11), stab cells 1 ± 0.4% (107 ± 43) and 0.5 ± 0.06% (54 ± 6), polymorphonuclear neutrophils 34 ± 2.1% (3677 ± 221) and 36.8 ± 1.8% (3985 ± 170), lymphocytes 57 ± 3.1% (6156 ± 324) and 55 ± 2.8% (5981 ± 301), monocytes 7 ± 1.5% (756 ± 162) and 5 ± 1.48% (544 ± 162), respectively.

After injection of spinal cord antigen into the sensitized animals no significant change was observed in the early period in the white blood picture (Fig. 2). However, injection of the same dose of spinal cord antigen in the later stages of sensitization, starting with the 21st day, led to important changes. For instance, on the 21st day the total leukocyte count had fallen to 12,400 ± 1094, on the 33rd day to 6600 ± 1870, and on the 40th day to 11,750 ± 1650 (Fig. 2). This decrease in the total leukocyte count took place primarily on account of the neutrophils (the number of polymorphs on the 33rd and 40th days was 1026 ± 194 and 983 ±

23, respectively) and also of mononuclear cells (lymphocytes and monocytes). The decrease in the total leukocyte count and in components of the leukocyte formula on the 21st, 33rd, and 40th days of sensitization after injection of spinal cord antigen into the blood stream, compared with the corresponding indices before injection, was significant. The decrease in the number of polymorphs in the blood was significant also by comparison with their level in intact guinea pigs.

After injection of nonspecific liver antigen into the peripheral circulation no significant change in the total leukocyte count or composition of the leukocyte formula was observed at any time of sensitization compared with the results obtained before injection of the antigen or in intact guinea pigs.

In view of the fact that injection of specific spinal cord antigen into the blood stream of sensitized animals led to a sharp decrease in the number of granulocytes in the leukocyte formula, the reactivity of the granulocytes was studied more closely in experiments *in vitro* by the allergic alteration method.

Granulocytes of the sensitized animals of group 3, stained with acridine orange in the presence of specific spinal cord antigen *in vitro*, when examined under the luminescence microscope showed morphological alteration with signs of deformation of the cell and degranulation and lysis of the cytoplasm. The number of such cells (Fig. 3) in the late stages of sensitization (32nd and 40th days) was considerably greater (27.5 ± 2.89 and 29 ± 2.58%, respectively) than in the early stages of sensitization, i.e., on the 3rd, 14th, and 22nd days (when it was 11.4 ± 1.6, 12.2 ± 2.5, and 14.5 ± 2.03%, respectively). Meanwhile specific alteration was more marked (results are significant) on the 32nd and 40th days than the spontaneous alteration (10.4 ± 2.2 and 10.8 ± 2.9%) and alteration under the influence of nonspecific kidney antigen (13.6 ± 1.59 and 12.6 ± 1.73%, respectively).

On immunization of animals with an oily suspension of *B. pertussis* cells, a lasting lymphocytosis is thus observed starting with the 14th day of sensitization. The decrease in reactivity of the peripheral blood lymphocytes to spinal cord antigen *in vitro*, found by the writers previously, was evidently not due to a change in the absolute number of lymphocytes in the late stages of sensitization (33rd and 40th days). Most probably this decrease in reactivity of the peripheral lymphocytes in the period of clinical manifestations of EAE is connected with the disappearance of specifically sensitized lymphocytes from the circulation and their penetration into the tissue of the CNS.

On the other hand, the reactivity of the peripheral blood leukocytes to spinal cord antigen both *in vivo* and *in vitro* is shown by these experiments to be clearly defined in the late stages of sensitization; the corresponding changes affected mainly the granulocytes.

Since leukocytolysis *in vivo* and allergic alteration of granulocytes *in vitro* reflect the activity of circulating antibodies [6], it is not surprising that these phenomena take place at that period of development of pertussis EAE when antibrain antibodies are present in high titer in the animals' blood [4]. Moreover, it is during this clinical period of development of pertussis EAE that allergic reactions of delayed type are characteristically depressed [4], possibly on account of a phenomenon of transitory hypersensitivity of delayed type (the Jones–Mote reaction) [8].

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