In our opinion, the fact that the endorphin concentrations are directly proportional to the severity of the clinical manifestations of bronchial asthma, together with the sharp fall of the endorphin levels as the result of corticosteroid therapy are evidence that the system for synthesis and secretion of these compounds is involved in the pathogenesis of the disease.

However, on the basis of the results so far obtained, the possibility cannot be completely ruled out that the changes observed are a secondary effect, due to a stress reaction of the patient to the asthmatic attack [7, 9].

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FORMATION OF ANTIGEN-DEPENDENT NONSPECIFIC IMMUNOGLOBULIN PRODUCING CELLS IN MICE IMMUNIZED WITH TWO T-INDEPENDENT ANTIGENS

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Injection of an antigen into an animal, besides inducing antibody formation, at the same time casues a sharp increase in synthesis of antigen-dependent nonspecific immunoglobulins (NIG) and an increase in the number of cells forming them (NIGFC) [1, 3, 4]. It was shown previously that on injection of two T-dependent [6] or one T-dependent and another T-independent [1] antigen, the number of NIGFC formed in the mouse spleen is about equal to the total number of cells arising in response to the action of each antigen separately.

The aim of this investigation was to study how the number of NIGFC changes during simultaneous administration of two T-independent antigens.

EXPERIMENTAL METHOD

Experiments were carried out on female BALB/c and C3H/A mice weighing 12-14 g. Salmonella Vi antigen [2] and polyvinylpyrrolidone (PVP) with a molecular weight of 350 kilodaltons (kD/ PVP_{350}) [5] were used as the T-independent antigens. The Vi-antigen and PVP_{350} were injected intravenously into mice in doses of 1 and 0.25-1 μg per mouse respectively. The number of antibody-forming cells (AFC) [7] and the number of immunoglobulin-forming cells (IGFC) [9] in the animals' spleens were determined on the 4th day. The number of NIGFC was calculated as the difference between the number of IGFC and the number of AFC per 10^6 living cells. The results were expressed in the form (M ± m).

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TABLE 1. Effect of T-Independent Antigens on Formation of Antibodies and of Antigen-Dependent NIG in BALB/c Mice (M ± m)

Antigen	Number of AFC per 10^6 cells		Number of IGFC	Number of NIGFC
	To Vi-antigen	To PVP_{350}	Per 10^6 cells	Per 10^6 cells
—	0—1	0	1908±376	1908
Vi-antigen	180±65	0—1	5648±2362	5468
PVP_{350}	0—2	90±42	4930±1347	4840
Vi-antigen + PVP	181±68	139±94	6333±2198	6013

Legend. Results of 6 experiments in each of which pools of spleen cells obtained from 3 or 4 mice were used are given.

TABLE 2. AFC Production in Response to Simultaneous Immunization of Mice with Vi-Antigen and PVP_{350}

Line of mice	Number of AFC per 10^6 cells when different antigens were used as the test		
	Vi-antigen	PVP_{24}	Mixture of antigens (1:1)
BALB/c	98±22	54±16	132±23
C3H/A	237±122	657±68	840±108

Legend. Three mice of each line were used.

As test antigens for determination of AFC to Vi-antigen and to PVP_{350}, we used sheep's red blood cells (SRBC), sensitized with Vi-antigen or PVP with a molecular weight of 24 kD (PVP_{24}), by a modified method [8]. SRBC were washed off with buffered physiological saline (BPS), pH 7.2, and a 5% suspension of washed SRBC in BPS was mixed with an equal volume of a freshly prepared solution of tannin in BPS in a concentration of 50 µg/ml. The mixture was incubated at room temperature with stirring for 15 min and the SRBC were washed 3 times with BPS. A 5% suspension of tanninized SRBC was then mixed with equal volumes of solutions of Vi-antigen (1 µg/ml) or PVP_{24} (50 µg/ml) in BPS, incubated for 15 min at room temperature with occasional stirring, and then washed off three times with BPS. From the resulting sensitized SRBC 10% suspensions in physiological saline were prepared and used in the experiments. To obtain preparations stable at 4°C for 1 week, a freshly prepared working solution of tannin (made from a mother solution in a concentration of 1 mg/ml, kept at 4°C for 1-2 months) and freshly prepared working solutions of Vi-antigen and of PVP_{24} (also made up from mother solutions in a concentration of 1 mg/ml) had to be used.

EXPERIMENTAL RESULTS

Immunization of BALB/c mice with PVP_{350} in a dose of 0.25-1 µg per mouse led to the appearance of AFC to PVP in the animals' spleens. The maximum of the response, just as in experiments with Vi-antigen [1], was observed on the 4th-5th day. The number of AFC varied from 48 to 132 per 10^6 cells, on average 85 and 102 AFC per 10^6 splenocytes on the 4th and 5th day respectively. When calculated per spleen, these numbers are equivalent to about 11,000 AFC; this is twice or three times greater than results obtained by other investigators [5]. This may be due to certain differences in the times and method of determination of AFC to PVP.

An even stronger response to PVP_{350} was obtained by the use of C3H/A mice immunized with 1 µg of PVP_{350}. The number of AFC in their spleens reached 463 ± 139 ± 139 10^6 cells (results of experiments on 12 individual animals), which is almost an order of magnitude greater than the number of AFC found in BALB/c mice. Such a high level of response to PVP_{350} (and to T-independent type 2 antigens in general), so far as the writers are aware, was found for the first time. The use of C3H/A mice can evidently prove useful when mechanisms of action of T-independent antigens are studied. It must also be pointed out that the response to Vi-antigen in mice of this line also was greater than in BALB/c mice: on average 269 ± 89 AFC/10^6 cells (results obtained on 6 individual animals). However, because the previous experiments to study the effect of simultaneous injections of T-dependent and T-independent antigens on NIGFC formation were conducted on BALB/c mice [1], in the present experiments it was also decided to use mice of this line for subsequent comparison of results.

Besides induction of AFC formation to PVP, immunization of BALB/c mice with PVP_{350} caused a sharp increase in the number of cells forming antigen-dependent NIG (Table 1). Absence of a polyclonal stimulation effect was characteristic of this situation: The number of cells forming antibodies to SRBC or to VI-antigen in response to injection of PVP_{350} did not exceed (1-3) 10^6 splenocytes, i.e., it was the same as in the background.

We know that PVP_{350} is a type 2 T-independent antigen, with no mitogenic effect [10]. The results fully agree with these data. The increase in the number of NIGFC in the absence of polyclonal stimulation indicate that this process is unconnected with mitogenic activity of the T-independent antigens used.

It is an interesting fact that immunization of the animals with Vi-antigen, which led to an increase in the number of NIGFC (Table 1), likewise did not give polyclonal stimulation; the number of AFC to SRBC or to PVP_{350} on the 4th day of immunization with Vi-antigen did not exceed $(1\text{-}4)/10^6$ spleen cells, i.e., it was the same as in the background. Vi-antigen was not identified as belonging to type 1 or type 2 of T-independent antigens. The fact that Vi-antigen did not give a polyclonal stimulating effect, which is characteristic of type 1 T-independent antigens [10], suggests that, like PVP_{350}, it is a type 2 antigen. This is also indicated by earlier data showing enhancement of the immune response to Vi-antigen by antisuppressor serum [2].

Establishment of the fact that PVP, like Vi-antigen, induces an increase in the number of NIGFC made it possible to proceed to the next stage, namely to an examination of how their formation is affected by simultaneous injection of both antigens. For this purpose the the animals (3 or 4 mice in a group) were immunized with Vi-antigen, with PVP_{350}, or with Vi-antigen + PVP_{350}, and on the 4th day the number of AFC to each of the antigens and the number of NIFC were determined in the spleens of the mice. Intact animals served as the control. The results are given in Table 1. Both Vi-antigen and PVP_{350} separately were found to induce AFC formation, accompanied by a marked increase in the number of NIGFC. Their number was usually 2-3 times greater than initially. Meanwhile summation of the effect in response to simultaneous injection of both antigens was not observed. These results differ from those obtained on simultaneous injection of two T-dependent [6] or of one T-independent antigens [1]. Summation of the effects observed in the previous experiments suggest that two different cell populations, each "triggered" by its own antigen, participate in antigen-induced NIGFC formation. The absence of summation, according to this argument, is evidence that Vi-antigen and PVP_{350} stimulate the same population of B-cells to form antigen-dependent NIGFC. The mechanism of this phenomenon is not clar. Two alternative hypotheses were suggested. First: Vi-antigen and PVP_{350}, if injected simultaneously into an animal, induce the same cells to form antibodies (and, correspondingly, to form antigen-dependent NIG). It follows from this hypothesis that AFC appearing as a result of immunization with both antigens ought to produce antibodies against both Vi-antigen and PVP_{350} simultaneously, which is extremely unlikely. Nevertheless, to test this hypothesis a special experiment was set up in which BALB/c mice (with low response) and C3H/A mice (with high response) were immunized with Vi-antigen and PVP_{350} simultaneously. SRBC sensitized with Vi-antigen, SRBC sensitized with PVP_{24}, and a mixture (1:1) of these red cells were used as test antigens. It was postulated that if antibodies to both antigens are produced by the same cells, when a mixture of test antigens is used the number of AFC will not differ from that found when each test antigen is used separately. Conversely, if antibodies to Vi-antigen and to PVP_{350} are formed by different cells, when a mixture of test-antigens is used summation of the number of AFC ought to be observed.

The data given in Table 2 show that the number of AFC in tests with a mixture of antigens was equal to the sum of AFC to Vi-antigen and of AFC to PVP_{350} (in both BALB/c and C3H/A mice). The hypothesis that antibodies to Vi-antigen and to PVP_{350} are synthesized by the same cell (and, correspondingly, that both antigens trigger the same NIGFC population), as would be expected, was not confirmed.

The alternative hypothesis is that NIGFC formation is brought about by certain factors secreted by antigen-activated B-cells (or by a few T-helper cells which participate in the response). We know that type 2 T-independent antigens can induce a response only in a population of $Lyb\text{-}5^+$ cells [5]. It can be tentatively suggested that antigen-dependent NIG in these cases also can form only $Lyb\text{-}5^+$ cells. Since this subpopulation accounts for only a certain part of all the B-cells [11], it can be suggested that B-cells, activated by one T-independent antigen, produce a nonspecific activating factor in sufficient amounts to exhaust the pool of cells potentially sensitive to it. The experimental verficiation of this hypothesis will be the aim of a subsequent investigation.

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RESPONSE OF RAT LUNG MACROPHAGES TO ZYMOSAN STIMULATION

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The course of an inflammatory process in the lung and realization of the mechanisms of defense of the lung tissue against injury are largely determined by reactivity of the lung macrophages. Not only their ability to ingest, kill, and digest microorganisms, but also the formation of a series of bactericidal principles in activated forms of macrophages, namely H_2O_2, the superoxide anion O_2^-, and singlet oxygen $^1O_2$ [4], is important under these circumstances. Secretory products of lung macrophages, on the one hand, modify the resistance of the host to infection (lysozyme, components C_2 and C_4 of complement, interferon, etc.), and on the other hand, they degrade components of the connective-tissue matrix of the lung: elastase, collagenase, enzymes catabolizing proteo- and glycosaminoglycans [1, 8]. In an inflammatory process lung macrophages cooperate with lymphocytes and fibroblasts [5]. Secretion not only of phlogogenic agents, but also of mediators of intercellular interactions, depends on reactivity of the macrophages. Accordingly it is important to study the response of the pulmonary component of the mononuclear phagocyte system (MPS) to the entry of agents activating macrophages and inducing inflammatory processes into the body. The yeast of polysaccharide zymosan is considered to be a stimulator of macrophages. If injected *in vivo* it causes a series of characteristic macrophage-dependent changes in the liver and lungs [3, 7].

The aim of the present investigation was to make a differential count of cells from broncho-alveolar washings and of loaded activated macrophages from the interstices of the lung, and to assess the general ingestive function of MPS after a single stimulation by zymosan.

EXPERIMENTAL METHOD

Experiments were carried out on 68 male Wistar rats weighing 200-250 g. The experimental animals were given a single intravenous injection of zymosan suspension in a dose of 0.1 mg/g body weight in 1 ml of 0.85% NaCl solution, and the control animals received an injection of 1 ml of 0.85% NaCl solution only. The animals were decapitated 2, 5, 7, 9 and 14 days after injection of zymosan, with simultaneous compression of the trachea. The lungs were weighed and immersed in physiological saline. The air remaining in the lungs was pumped out through a cannula introduced into the trachea, after which medium 199 with heparin (5 U/ml) was injected in a dose of 5 ml/g weight of the lungs at the rate of 0.2 ml/sec. After 30 sec

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