determine the decrease in antibody production with time after injection of an antigen. The decrease in the number of AFC observed in response to SRBC in a dose of 2×10^9, against the background of marked stimulation of proliferative activity of the hematopoietic stem cells, can evidently be taken to indicate that the above mechanism plays a definite role in the development of tolerance to this particular antigen.

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CHANGES IN THE PROLIFERATIVE RESPONSE OF HUMAN LYMPHOCYTES IN VITRO UNDER THE INFLUENCE OF SUBCELLULAR COMPONENTS OF *Bordetella pertussis*

P. V. Glan, A. N. Mats, and A. A. Armarchuk

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Different subcellular components of *Bordetella pertussis* were found to have a similar inhibitory action on incorporation of thymidine-^{3}H by lymphocytes stimulated by phytohemagglutinin. Lymphocytes were obtained from donors immunized with tetanus toxoid. However, the same components of *B. pertussis* had a differential action on lymphocyte proliferation in the presence of tetanus toxoid: murein-containing membranes increased incorporation of thymidine-^{3}H, the RNA-containing fraction inhibited it, and the water-soluble components of the homogenate had no effect on lymphocyte proliferation.

KEY WORDS: *Bordetella pertussis*; proliferation of lymphocytes; tetanus toxoid.

During contact between mammalian cells and subcellular components of *Bordetella pertussis* in vitro various changes take place in the surface membrane structures and, in particular, the architectonics of the surface of tumor cells in culture is modified [5], macrophages lose their ability to form erythrocytic rosettes [7], and lymphocytes are stimulated toward nonspecific blast transformation [3]. Consequently it might be expected that after exposure to the action of subcellular components of *B. pertussis* the ability of human lymphocytes to respond by proliferation to lectins and specific antigens in vitro would also be changed.

The object of this investigation was to study the character of these changes during the response to phytohemagglutinin (PHA) and tetanus toxoid.

Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.)

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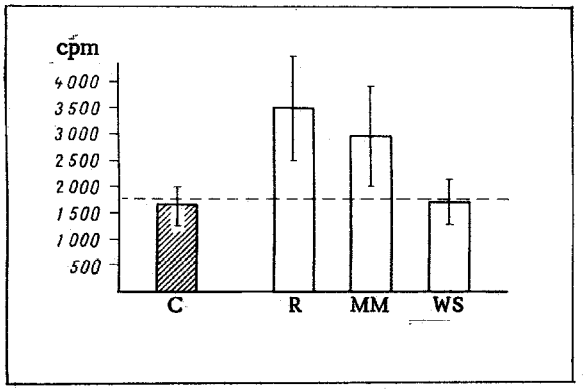

Fig. 1

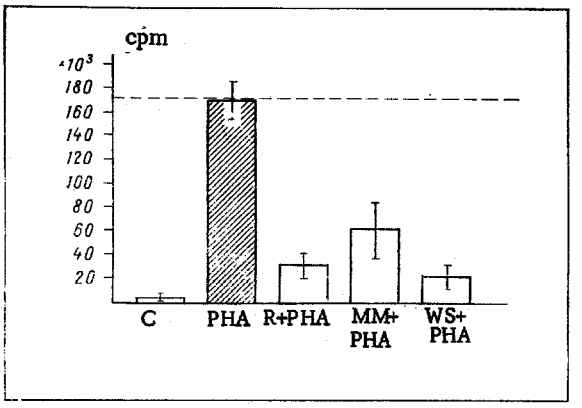

Fig. 2

Fig. 1. Action of preparations of *B. pertussis* (50 μg/ml) on spontaneous DNA synthesis in lymphocyte culture. Here and in Figs. 2 and 3: C) control; R) RNA-containing particles; MM) murein-containing membranes; WS) water-soluble components. Ordinate, incorporation of thymidine-^{3}H (in cpm).

Fig. 2. Changes in proliferative response to PHA under influence of *B. pertussis* preparations.

EXPERIMENTAL METHOD

B. pertussis cells grown on casein-carbon agar were disintegrated in the frozen state in an Adebo X-press and fractionated by differential centrifugation. Fragments of the cells were treated with sodium dodecylsulfate, digested with pepsin, trypsin, and lysozyme, and washed to give a preparation of murein-containing supporting membranes [10]. RNA-containing particles were sedimented from the supernatant of the disintegrated mass after centrifugation at 20,000 g for 1 h with the aid of dihydrostreptomycin sulfate [1]. The remaining supernatant contained water-soluble components of the extract. The three subcellular fractions of *B. pertussis* thus obtained possessed different biological properties [2]. The preparation of RNA-containing particles protected mice against intracerebral *B. pertussis* infection, the membrane preparation possessed marked adjuvant activity associated with low toxicity, and the fraction of water-soluble components of the extract possessed considerable toxicity.

Leukocytes of blood donors immunized with tetanus toxoid were isolated from blood using 10% gelatin solution and incubated for 30 min at 37°C with subcellular components of *B. pertussis* and then washed and cultured for 72 h in medium No. 199 with 10% human serum (group IV) in the presence of PHA (10 μg/ml) or for 144 h in the presence of tetanus toxoid (0.2 fixation unit/ml). The leukocytes were cultured in centrifuge tubes, each of which contained 2 million cells in 2 ml culture medium. The intensity of the blast-transformation reaction was assessed by incorporation of thymidine-^{3}H which was added to the culture medium 24 h before the moment of fixation, in a concentration of 1 μCi/ml. The contents of each tube was transferred to a micropore filter and the cells were washed with physiological saline and 5% TCA. The filters were then transferred to flasks with scintillation fluid for radiometry.

EXPERIMENTAL RESULTS

The action of the subcellular components of *B. pertussis* on DNA synthesis was first studied in unstimulated cultures. In a dose of 50 μg/ml dry weight, none of the fractions was found to inhibit incorporation of thymidine-^{3}H. The RNA-containing particles and membranes were actually found to possess a weak mitogenic action (Fig. 1).

It was next found that contact between lymphocytes and the three fractions of subcellular components of *B. pertussis* for 30 min inhibited blast transformation in response to PHA. All three fractions possessed significant ($P < 0.01$) and about equal inhibitory action (Fig. 2). In the same experiments a parallel study was made of the effect of preliminary treatment with subcellular components of *B. pertussis* on lymphocyte proliferation induced by tetanus toxoid. A significant difference was found in the action of the three fractions.

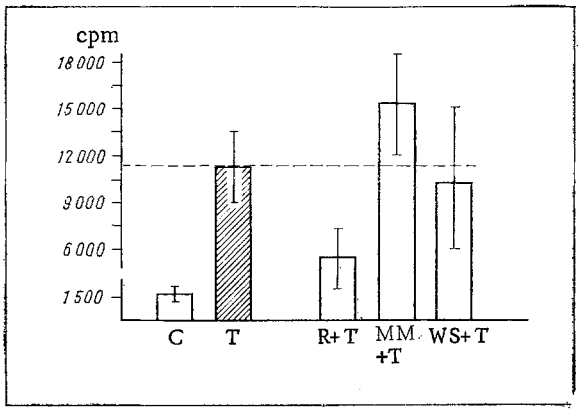

Fig. 3. Changes in proliferative response to tetanus toxoid (T) under the influence of preparations from *B. pertussis*.

The fraction of RNA-containing particles inhibited the response of the lymphocytes by about half. The membrane fraction, on the other hand, had a stimulating action. The fraction of water-soluble components caused no change (Fig. 3).

The experiments thus showed that these subcellular components of *B. pertussis* can modify the proliferative response of human lymphocytes to PHA and tetanus toxoid. Significant inhibition of the response of that lymphocyte population (probably thymus-dependent lymphocytes) that is stimulated by PHA was found. These results are in agreement with data in the literature on inhibition of the reactions of cellular immunity in vivo by *B. pertussis* (for example, inhibition of reactions to tuberculin [8], abolition of the graft versus host reaction [6], or depression of transplantation immunity [4] which are dependent on the functions of thymus-dependent lymphocytes).

On the other hand, the same subcellular components of *B. pertussis* had different effects on the specific proliferative response of the lymphocytes to tetanus toxoid, for which the functions of both T- and B-lymphocytes are probably responsible [9]. The stimulating effect of the membranes of *B. pertussis* can be regarded as an in vitro reflection on their immunoadjuvant properties manifested in vivo. In lymphocyte cultures they can either abolish the action of T-suppressors or stimulate T-helpers, or again, they may activate B-cells directly.

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