The compounds tested likewise had no cytotoxic action in concentrations 5 times greater than the maximal concentration used in the experiments. Differences found were statistically significant (by Student's criterion).

It can thus be concluded from these results that diphosphonates have a protective action on the outer cell membrane of lymphocytes against immunologic injury. This fact may be of great importance to the understanding of the mechanisms of development and the treatment of diseases in which the leading pathogenic factor is a disturbance of the integrity of the outer cell membrane on an immunologic basis.

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STATE OF NONSPECIFIC RESISTANCE IN GERMFREE AND *Escherichia coli* CONTAMINATED MINIATURE PIGLETS

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The phagocytic activity of the leukocytes and the serum complement, properdin, and lysozyme levels were studied in germfree miniature piglets and similar animals contaminated with *Escherichia coli* 055 and *E. coli* 083. In the presence of autologous serum and complement phagocytosis of *E. coli* 055 cells was inhibited, but it was considerably intensified under the influence of specific opsonins (antibodies against *E. coli* 055). Lowered levels of complement, properdin, and lysozyme were found in the germfree animals. After peroral monocontamination with *E. coli* the formation of properdin and complement was stimulated the most, and that of lysozyme the least. Antibodies against *E. coli* 055 were not found in the monocontaminated piglets. The highest lysozyme levels were found in the previously germfree animals, which points to the role of other contamination factors than *E. coli* cells in the stimulation of lysozyme. It is concluded that microbial contamination plays an important role in the development of the cellular and humoral factors of resistance.

KEY WORDS: gnotobiotic miniature piglets; phagocytosis; complement; properdin; lysozyme.

With the development of the gnotobiological approach the attention of research workers has been increasingly attracted to the study of the role of the microbial factor in the formation of the immunobiological reactivity of the host. Experiments on germfree animals of various species have yielded new data on the role of the microflora and its individual representatives in the mechanisms of immunogenesis and also of nonspecific resistance of the host to infection. Meanwhile, the role of the microbial factor in the formation of resistance has still been only inadequately studied. Germfree piglets are particularly valuable for the study of the microbial influence on the development of immunobiological reactivity and nonspecific resistance to infection, for because of the nature of the structure of their placenta (of the chorioepithelial type), no globulins of maternal origin can enter the fetuses [9].

Methods of obtaining and rearing germfree miniature piglets were developed and introduced at the Research Laboratory of Experimental Biological Models, Academy of Sciences of the USSR, beginning in 1975.

Research Laboratory of Experimental Biological Models, Academy of Sciences of the USSR, Moscow Region. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. N. Zhukov-Verezhnikov.)

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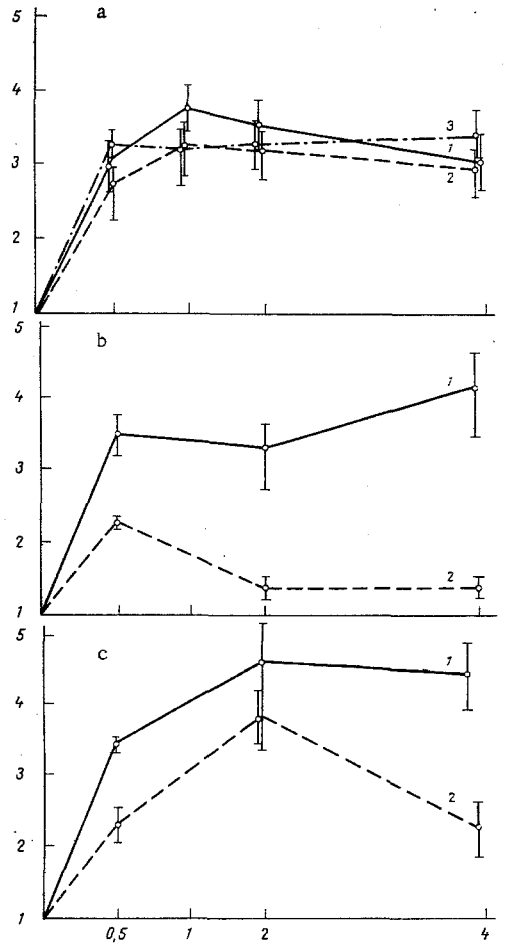

Fig. 1. Phagocytosis of E. coli 055 cells by leukocytes of monocontaminated and germfree miniature piglets. Abscissa, duration of phagocytosis (in h); ordinate, log of number of living microorganisms in 10^6 leukocytes. a) **Phagocytosis in piglets** monocontaminated with E. coli 055: 1) in presence of autologous serum of these piglets, 2) in presence of complement, 3) in Hanks' medium; b) phagocytosis in piglets monocontaminated with E. coli 083: 1) in presence of autologous serum of these piglets, 2) in presence of specific antiserum against E. coli 055; c) phagocytosis in germfree piglets: 1) in presence of autologous serum of these piglets, 2) phagocytosis in presence of specific antiserum against E. coli 055.

The object of this investigation was to study the state of nonspecific resistance in germfree miniature piglets and also in the same animals when contaminated with bacteria of the enteropathogenic strain Escherichia coli 055 and the nonpathogenic strain E. coli 083.

EXPERIMENTAL METHOD

Germfree miniature piglets were obtained by hysterotomy in an operating isolator of the writer's own design [3]. The canine teeth of the newborn animals were extracted, after which the resulting germfree piglets

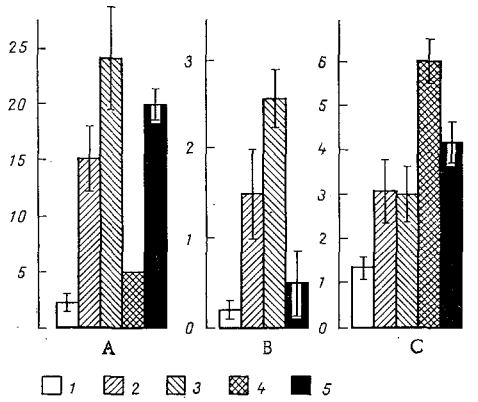

Fig. 2. Humoral indices of nonspecific immunobiological reactivity in gnotobiotic and ordinary piglets. A) Complement (in units/ml); B) properdin (in units/ml); C) lysozyme (in μg/ml); 1) germfree piglets; 2) piglets monocontaminated with E. coli 083; 3) piglets monocontaminated with E. coli 055; 4) former germfree piglets; 5) ordinary piglets.

were transferred to an isolator for rearing. The animals were fed on a milk diet with the addition of vitamins and trace elements [10]. A microbiological control was set up as described in [2]. All the technological processes of the gnotobiological experiment were carried out in accordance with the rules elaborated in [7].

Miniature piglets aged 2.5 months – germfree, monocontaminated with E. coli and ordinary – were used. Enteropathogenic strain E. coli 055 and nonpathogenic strain E. coli 083, obtained from the Institute of Microbiology, Czechoslovak Academy of Sciences (Prague), were used for monocontamination. The piglets were contaminated at the age of 1.5 months per os in a dose of 5-10 billion E. coli cells.

The development of nonspecific resistance in the gnotobiotic animals was assessed from the indices of phagocytic activity of the blood leukocytes and humoral factors of immunobiological reactivity (complement, properdin, lysozyme). Humoral factors also were studied in piglets of the same age, removed at the age of 1.5 months from a sterile isolator to the ordinary microbial environment, in which they remained for one month before the experiment. Each group studied contained 6 to 8 animals.

The phagocytic activity of the leukocytes from germfree and monocontaminated piglets was determined in vitro by a modified method [8]. The object for phagocytosis consisted of E. coli 055 cells, in the ratio of one bacterium to 50 leukocytes. To study the development and role of opsonizing factors in phagocytosis, the phagocytic activity of the leukocytes was compared in the presence of serum from germfree and monocontaminated piglets, and also of nonspecific and specific opsonins (a standard preparation of complement and antiserum against E. coli 055 in a dilution of 1:10). Under these conditions the phagocytic activity of the leukocytes of germfree and monocontaminated animals was determined.

After incubation for 30 min to destroy extracellular bacteria, a mixture of antibiotics (100 i.u. penicillin and 100 μg streptomycin) was added to the phagocytic system. Samples of the phagocytic mixture were taken 30 min, and 1, 2 and 4 h after the beginning of incubation. The cell residue, washed in physiological saline, was disintegrated by pipeting in 1 ml of sterile distilled water. Tenfold dilutions were prepared from the resulting cell lysate and 0.5 ml of each dilution was seeded on Endo-agar in petri dishes. The number of colonies growing on the dishes was counted after incubation for 24 h and the number of living E. coli cells present in 10^6 leukocytes was calculated.

The serum properdin and complement levels were determined by the method adopted at the Institute of Pediatrics, Academy of Medical Sciences of the USSR [4]. Lysozyme in the serum was determined by a dish method using a test culture of Micrococcus lysodeicticus. The specific immune response in piglets monocontaminated with E. coli 055 was studied by determining the levels of antibodies against these microorganisms by the passive hemagglutination test.

EXPERIMENTAL RESULTS

Reduced phagocytic activity of the blood leukocytes of the germfree and monocontaminated piglets was discovered in the presence of the serum of these animals (Fig. 1). On the addition of complement to the phagocytic test system, no increase in phagocytosis was found either in the germfree or monocontaminated animals. When antiserum against E. coli 055 was used as the opsonin, a marked increase was observed in the phagocytic activity of the leukocytes, and it was especially marked in piglets monocontaminated with E. coli 055 (Fig. 1).

The absence of any opsonizing effect of serum from the monocontaminated animals coincided with the absence of antibodies against E. coli 055 in them. Whereas in ordinary control piglets the titer of normal antibodies against E. coli 055 was 1:32-1:64, in the germfree and monocontaminated animals none of these antibodies were found (antibodies were detected in only a few of the monocontaminated piglets in a titer of 1:2-1:4).

These results, indicating the great importance of specific opsonins (antibodies) for phagocytosis of pathogenic E. coli cells in vitro, are in full agreement with the dependence of the phagocytic activity of the mononuclear phagocytic system in germfree animals on the presence of specific opsonins in vivo discovered previously [6].

A study of the humoral indices of nonspecific immunobiological reactivity in the germfree piglets compared with ordinary animals showed lower levels of complement and properdin. After monocontamination of gnotobiotic piglets a marked increase was observed in the indices of nonspecific resistance; the enteropathogenic strain E. coli 055, moreover, possessed a stronger stimulating effect than the nonpathogenic strain E. coli 083 (Fig. 2). The properdin levels in the monocontaminated piglets were much higher than in ordinary animals. The results demonstrate the important role of the microbial factor as a whole and of E. coli in particular in the stimulation of complement and properdin formation.

The pathogenic and nonpathogenic strains of E. coli also stimulated lysozyme formation to some extent, and equally. However, the lysozyme concentration was higher in ordinary animals. The lysozyme concentration was particularly high in the serum of former germfree piglets. Comparison of the lysozyme levels in all categories of animals, depending on their microbial status, suggests that E. coli cells play an important, but not the principal role in the stimulation of this factor of nonspecific resistance under the influence of microflora. This role can be performed more successfully by other microorganisms or by their associations as well as by other as yet unidentified factors which, directly or indirectly (through leukocytosis) stimulates lysozyme formation. It is interesting to note that the highest concentration of lysozyme was discovered in the former germfree animals which, against the background of a general deficiency of immunity, were exposed simultaneously to intensive and varied microbial action. The results emphasize the special importance of this factor of nonspecific resistance for the immunologically intact organisms exposed to massive microbial action. Considering the important role of lysozyme in resistance to infection, especially in children [5], the importance of the further study of how its formation depends on the microbial factor will be evident.

The enhancement of all factors of nonspecific resistance during contamination of germfree animals evidently reflects the stress response of the host to microbial action and is adaptive in character. Under these circumstances the development of the individual indices of nonspecific resistance in germfree animals on monocontamination with cells of the genus Escherichia depends on the pathogenicity of the microorganisms. These observations deepen our knowledge of the different effects of other microorganisms present in the normal microflora of the host on the indices of nonspecific resistance.

On the whole, the results demonstrate the preferential development of nonspecific factors of resistance in animals during monocontamination with E. coli in the absence of any accompanying microflora.

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EFFECT OF STEM CELL INHIBITION FACTOR AND MACROPHAGE MICRATION INHIBITION FACTOR ON EXOCOLONIZATION AND MIGRATION OF MOUSE SPLEEN CELLS

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Hematopoietic stem cell inhibition factor (SCIF), liberated by lymphocytes treated with antilymphocytic globulin, causes inhibition of migration of the spleen cells of intact mice. The degree of inhibition of migration corresponds to the colony-inhibiting activity of the SCIF. Macrophage migration inhibition factor (MMIF) obtained in the H-2 system has a stimulating effect on exocolonization in mice on treatment of a bone-marrow graft in vitro. The colony-stimulating activity of MMIF corresponds to its inhibitory effect on migration of spleen cells. Incubation of bone marrow cells with MMIF for 30 min is more effective than incubation for 2 h. It is suggested that SCIF and MMIF are not identical with one another.
KEY WORDS: Stem cell inhibition factor; migration inhibition factor; exocolonization; migration.

Investigation of the biological properties of hematopoietic stem cell inhibition factor (SCIF), which is secreted by lymphocytes treated with antilymphocytic globulin (ALG) [1], has shown that it has the ability to inhibit migration of the spleen cells of intact mice [3].

To study the possible identity of SCIF with marcrophage migration inhibition factor (MMIF), in the investigation described below the action of these factors on hematopoietic stem cells and migration of spleen cells was studied.

EXPERIMENTAL METHOD

SCIF was obtained by the method described earlier [2]. MMIF was obtained in an H-2 system by the method suggested by Friedman [6], in the modification of Suslov et al. [4].

The method of cloning hematopoietic stem cells in vivo in lethally irradiated recipients [10] was used. The bone marrow cells intended for transplantation were treated in vitro with SCIF or MMIF for 120 or 30 min, after which they were washed three times with medium. An intravenous injection of $0.5 \cdot 10^5$ cells of intact or treated bone marrow was given to the recipients 4 h after irradiation (830 rad). The animals were killed nine days later and the number of macroscopically visible colonies on the surface of the spleen was counted.

To test the activity of the resulting MMIF and to study the biological properties of the SCIF, the capillary method of macrophage migration was used [5]. Cells from the intact spleen of (GBA × C57BL/6) F_1 mice were used as migrating cells. The degree of migration was assessed by weighing the zone of migration, traced on

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