ORIGIN OF CELLS REDUCING NORMAL HEMOLYSINS IN GUINEA PIGS

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Cells producing normal antibodies against sheep's erythrocytes were revealed by local hemolysis in agar in the spleen of embryos in the late stage of development and of newborn guinea pigs. Preliminary immunization of the pregnant animals with sheep's red cells did not affect the number of hemolysin-producing cells in the spleen of the embryos or newborn guinea pigs. The number of normal antibody-forming cells in the spleen of sterile adult guinea pigs was the same as in animals of the same species kept under ordinary conditions. The results suggest that normal hemolysin-producing cells arise in guinea pigs not by antigenic stimulation through cross-reacting antigens of the external environment, but by physiological differentiation of predetermined precursor cells. This hypothesis corresponds to one of the postulates of the clonal selection theory of immunogenesis.

KEY WORDS: normal antibody-forming cells; embryos; germ-free animals.

So-called normal (natural) antibodies against antigens of different origin are often found in the blood serum of nonimmunized animals and man [22]. These antibodies have been found also against sheep's red cells [4, 6, 11]. Normal antibody-forming cells (AFCs) have been demonstrated by the method of local hemolysis in gel, even when antibodies could not be determined quantitatively in the blood, in the spleen, and other organs of mice [8, 11, 16, 21], rats [17], rabbits [14, 18], hamsters [3], piglets [18], birds [9], and man [7]. The writers have described differences in the number of these cells in animals of different species [3].

On the question of the origin of normal antigens (and, correspondingly, of AFCs) the most commonly held view is that they arise as a result of natural immunization of animals with cross-reacting antigens [19, 22]. In particular, the Forssman antigen, found in sheep's red cells, is widely distributed in the environment – in bacteria and food products – possible materials giving rise to spontaneous immunization of animals not normally possessing this antigen (rats, rabbits, pigs).

According to another view, shared by fewer investigators, normal antibodies arise as a result of physiological differentiation of precursor cells into antibody-forming cells unconnected with any specific antigenic stimulus. This view is based chiefly on indirect evidence; the simultaneous appearance of normal antibodies against different antigens [12], the high resistance of normal AFCs to factors depressing the immune response – such as irradiation [4, 11], hyperimmune serum [21], antilymphocytic serum [15], and so on.

One of the chief arguments put forward in support of the "immune" nature of normal antibodies is the absence of normal AFCs in newborn mice, rabbits, and pigs, in which these cells begin to appear 1-2 weeks after birth, together with the fact that in germ-free animals (mice, pigs) cells producing antibodies against sheep's red cells are either completely absent or appear much later and in smaller numbers than in ordinary animals [10, 16, 18, 20].

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In the investigation described below, the presence of normal AFCs was investigated by using sheep's red cells as antigen, in guinea pigs in the early stages of development, and also in germ-free guinea pigs and guinea pigs kept under ordinary conditions. Guinea pigs were chosen as the test object for the following reasons: 1) unlike many other species of laboratory animals, guinea pigs mature early (they can see at birth); 2) the organs of guinea pigs contain Forssman antigen, as a result of which it cannot induce the formation of normal antibodies against sheep's red cells in the animals of this species.

EXPERIMENTAL

Experiments were carried out on guinea pig embryos (taken 1-2 days before the expected day of birth, as revealed by the degree of separation of the pubic symphysis in the pregnant animals), newborn animals (during the first 24 h after birth), and adult guinea pigs aged 1-3 months.* The material chosen for study was the spleen, in which the number of hemolysin-producing cells was determined by the "direct" method of local hemolysis in agar [2, 13]. The identification and counting of the zones of hemolysis formed in agar were carried out macroscopically and microscopically (dry objective, 40 × or 70 ×) from the presence of a nucleated cell in the center of a zone of hemolysis. Statistical analysis of the results was carried out with the aid of Student's t-test and Wilcoxon–Mann–Whitney nonparametric U-test [1].

RESULTS

The data relating to the presence of AFCs in the embryonic and newborn guinea pigs are given in Table 1.

The results show that hemolysin-producing cells were found more often in the newborn guinea pigs than in the embryos ($P = 0.032$). However, their relative and absolute numbers in the spleens of those animals in which they were found were practically identical. The writers showed previously [3] that in adult guinea pigs the number of normal AFCs per 10^6 nucleated spleen cells is 0.15 (0.11-0.21). Comparison of this value with the data in Table 1 shows that the relative number of AFCs in the embryos and the newborn guinea pigs did not differ significantly from that in the adult animals (although in the adult guinea pigs, unlike the younger animals, normal AFCs were found in every case and their absolute number was greater because of the much larger size of the spleen). In the next series of experiments the effect of immunization of the pregnant guinea pigs with sheep's red cells on the number of AFCs in the spleen of the embryos and newborn animals was studied. For this purpose, the females in the late stage of pregnancy were given an intravenous injection of $1 \cdot 10^9$ sheep's red cells, and 5-8 days later the spleen was taken for investigation from the embryos (1-2 days before birth) and from the newborn guinea pigs (during the first 24-48 h of life). The results are given in Table 2.

As the results of Table 1 and 2 show, immunization of the pregnant females had no effect either on the frequency of detection of AFCs in the embryos and newborn animals or on their number in the spleen.

In the experiments on intact adult animals guinea pigs of the following types were used: 1) sterile (germ-free) guinea pigs receiving sterile food; 2) ordinary guinea pigs, also receiving sterile food; 3) ordinary guinea pigs which for 1-3 weeks before sacrifice received sterile food to which feces were added in

* The materials taken from adult germ-free and the corresponding control animals were generously provided by O. V. Chakhava, Head of the Laboratory of Gnotobiology, N. G. Gamaleya Institute of Epidemiology and Microbiology, and the writers wish to express their gratitude to him.

TABLE 1. Number of Normal AFCs in Spleen of Embryos and Newborn Guinea Pigs (×g and 95% confidence limit)

Test object	Number of animals investigated	Number of animals in which AFCs found	Number of AFCs	
			pigs (per 10^6 spleen cells)	per spleen
Embryos	11	4	0,11 (0,04—0,29)	5 (0,7—27)
Newborn animals	20	15	0,11 (0,07—0,16)	7 (4—10)

TABLE 2. Number of AFCs in Spleen of Embryos and Newborn Guinea Pigs after Immunization of Pregnant Females with Sheep's Red Cells (×g and 95% confidence limit)

Test object	Number of animals investigated	Number of animals in which AFCs found	Number of AFCs	
			per 10^6 spleen cells	per spleen
Embryos	14	6	0,11 (0,08—0,15)	7 (5—10)
Newborn animals	14	11	0,13 (0,07—0,25)	7 (4—14)

TABLE 3. AFCs in Spleen of Adult Nonimmune Guinea Pigs Kept under Different Conditions

Animals	Number of AFCs in individual guinea pigs (per 10^6 spleen cells)
Germ-free, receiving sterile food	0,06 0,19 0,20 0,60 0,66 0,82
Ordinary, receiving sterile food	0,07 0,21 0,66 0,94
Ordinary, receiving sterile food + feces	0,13 0,14 0,20

Legend. Differences in numbers of AFCs in animals of various groups not significant ($P > 0.05$) by the use of the t- and U-tests

order to simulate bacterial stimulation of the intestine. Determination of the number of AFCs in the spleen of the animals in these groups showed no difference between them.

The first point to note when summing up these results is that normal hemolysin producing cells are present in the spleen not only of newborn guinea pigs but also of embryos in the late stage of development. The absence of any effect of immunization of the mothers with sheep's red cells on the number of AFCs in the fetuses and newborn guinea pigs is evidence merely that the antigenic material* circulating in the maternal organism does not reach the embryo or that the immunocompetent cells of the embryo at that particular stage of the development do not give an immune response to erythrocytic antigen. Hence it can be concluded that the normal AFCs found in the spleen of guinea pigs embryos and also of the newborn animals during the first 24-48 h do not arise as a result of antigenic stimulation by antigens (bacteria, food) penetrating into the maternal organism. This conclusion is supported by data showing that the number of AFCs in the spleen of germ-free guinea pigs is indistinguishable from that in ordinary animals.

On the whole, the results are in agreement with one of the propositions of the clonal selection theory [5], which postulates that cells predetermined toward various antigens and capable, at a certain stage of development, of producing normal antibodies, are present in animals.

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*As well as intact red cells this material could also be their in vivo breakdown products, as was shown by earlier experiments on mice.