Allowing for this hypothesis and also for data showing that chalones obtained from newborn skin may have an inhibitory effect on proliferation of the epidermis in adult animals [11], whereas chalones isolated from mature skin act only on the definitive tissue [5], it can be postulated that in the course of histogenesis of the epidermis maturation of the G_2 chalone molecule takes place. Evidence in support of this hypothesis is given by data showing that chalones isolated from the skin of newborn and adult animals differ in molecular weight and in certain properties of their action [12]. To judge from the data described above, they have common antigenic determinants, for chalone was found in the embryonic epidermis with the aid of antibodies obtained to G_2 inhibitor from adult skin.

In the process of histogenesis of the epidermis of the rat skin reversal of the localization of G_2 chalone is thus observed. In embryos aged 17-21 days and in newborn animals it is absent in the basal layer, on the 2nd-5th days of postnatal development it is found in all layers of the epidermis, but on the 6th-9th days it begins to be found chiefly in spinous and basal cells. In other words, together with the completion of histogenesis of the epidermis, the distribution of G_2 chalone characteristic of mature tissue also is established.

LITERATURE CITED

1. A. P. Dyban et al., in: Objects in Developmental Biology [in Russian], Moscow (1975), pp. 505-566.
2. A. A. Zavarzin, DNA Synthesis and the Kinetics of Cell Populations in Mammalian Ontogeny [in Russian], Leningrad (1967).
3. S. A. Ketlinskii, Arkh. Anat., No. 1, 29 (1980).
4. S. A. Ketlinskii, Arkh. Anat., No. 2, 90 (1981).
5. S. A. Ketlinskii, "Tissue-specific regulation of cell proliferation and differentiation under normal and pathological conditions," Author's Abstract of Doctoral Dissertation, Leningrad (1981).
6. S. A. Ketlinskii and A. S. Simbirtsev, Arkh. Anat., No. 6, 58 (1981).
7. F. M. Letuchaya and S. A. Ketlinskii, Tsitologiya, No. 2, 176 (1980).
8. F. W. Bauer, Dermatologica, 145, 16 (1972).
9. W. S. Bullough, Cancer Res., 25, 1683 (1965).
10. J. Hanson, J. Anat., 81, 174 (1947).
11. J. M. Marrs and J. J. Voorhees, J. Invest. Derm., 56, 353 (1971).
12. S. Rothberg and B. C. Arp, Natl. Cancer Inst. Monogr., 38, 93 (1973).

ACTION OF PERTUSSIS VACCINE ON MOUSE HEMATOPOIETIC STEM CELLS

V. V. Khorobrykh, N. S. Barteneva, A. V. Sanin, and A. E. Snegireva

UDC 615.371:579.841.94].015.44:612.419

KEY WORDS: pertussis vaccine; stem cells.

The immunostimulating properties of many adjuvants have now been studied in detail. Microbial endotoxins, Freund's complete adjuvant (SCA), and pertussis vaccine are well known and widely used. However, much remains unexplained in respect of the mechanisms of the stimulating action of adjuvants on the immune response. Immunogenesis and hematopoiesis are intricately interconnected. However, whereas many aspects of the effect of bacterial endotoxins and FCA hematopoiesis have now been explained [8, 12, 13], the action of pertussis vaccine on hematopoietic cells has virtually not been studied.

The object of this investigation was to study this problem, which may be important for the development of approaches to the specific regulation of immunity against both infections and tumors.

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 6, pp. 107-110, June, 1982. Original article submitted December 3, 1981.

0007-4888/82/9306-0828$07.50

TABLE 1. Formation of Endogenous Colonies in Spleen of Mice Immunized with Pertussis Vaccine and Then Irradiated in a Dose of 5.5 Gy (M ± m)

Time of injection of vaccine (before irradiation)	Dose of vaccine, BC	Strain of mice			
		BALB/c	CBA	C57BL/6	(CBA×C57BL/6) F_1
4 h					
	Control	2,1±0,4 (14)	3,4±0,5 (10)		
	10^7	2,8±0,4 (10)	8,8±1,0*(16)	—	—
	10^{10}	15,4±0,7*(14)	10,6±0,9*(14)		
1 day					
	Control	1,6±0,5 (11)	3,3±0,5 (14)	3,0±0,4 (10)	3,0±0,8 (7)
	10^7	3,9±0,4*(13)	13,1±0,6*(16)	6,5±1,0*(11)	4,3±0,8 (7)
	10^{10}	18,7±1,8*(12)	16,4±0,6*(18)	22,5±2,4*(12)	21,8±3,6*(5)
3 days					
	Control	2,1±0,4 (16)	3,7±0,3 (14)	2,8±0,7 (10)	3,2±1,2 (6)
	10^7	4,1±0,5*(12)	18,8±0,9*(18)	6,4±1,0 (10)	6,8±1,5 (7)
	10^{10}	26,5±1,9*(11)	25,6±1,2*(18)	14,9±2,5*(11)	21,7±2,0*(7)
5 days					
	Control	2,9±0,6 (11)	3,3±0,2 (18)	3,1±0,5 (10)	3,6±1,1 (5)
	10^7	8,7±1,1*(12)	14,4±0,6*(20)	10,9±1,2*(13)	8,6±1,7 (5)
	10^{10}	12,0±1,5*(10)	5,9±0,4*(20)	6,9±1,3 (10)	14,6±2,1*(8)
9 days					
	Control	3,0±0,4 (20)	2,0±0,3 (18)	3,6±0,6 (10)	3,8±0,7 (8)
	10^7	11,3±1,3 (12)	6,4±0,2*(16)	7,8±1,2* (12)	8,1±1,4 (10)
	10^{10}	8,1±1,0 (14)	6,0±0,4*(14)	5,9±1,4 (10)	9,6±1,7*(9)
21 days					
	Control	3,3±0,4 (21)	2,9±0,3 (20)	3,1±0,5 (12)	3,8±0,4 (9)
	10^7	6,7±0,7*(21)	5,9±0,3*(18)	9,5±1,3*(10)	5,6±0,8 (8)
	10^{10}	4,0±0,7 (22)	3,8±0,2 (20)	4,2±0,8 (13)	6,9±1,3 (8)
30 days					
	Control	2,8±0,3 (16)	—	—	—
	10^7	3,0±0,4 (14)	—		
	10^{10}	3,2±0,5 (12)			

Legend. Number of mice in parentheses.
*Significance of differences compared with control ($P < 0.05$).

EXPERIMENTAL METHOD

Male BALB/c, CBA, and C57BL/6 mice and (CBA × C57BL/6)F_1 hybrids weighing 20-23 g were obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. Standard chilled vaccine from strain 305 of *Bordetella pertussis*, obtained from the Laboratory of Physiology and Biotechnology of Microbes, Moscow I. I. Mechnikov Research Institute of Vaccines and Sera, washed 3 times to remove formalin, was used for immunization. The vaccine was injected intravenously into mice in a dose of 10^7 or 10^{10} bacterial cells (BC) per mouse. To study endocolonization the mice were irradiated in a dose of 5.5 Gy at different time intervals after immunization. On the 9th day after irradiation the spleens were fixed in Bouin's solution and visible colonies were counted macroscopically. Hematopoietic cells were transplanted by the method in [14]: $5 \cdot 10^4$ syngeneic bone marrow cells or $5 \cdot 10^5$ spleen cells were injected intravenously into lethally irradiated (7.5 Gy) CBA mice. The mice were killed 8 days after transplantation, the spleens were fixed in Zenker's fluid, and colonies were planted. No colonies developed in the spleen of lethally irradiated mice (36 animals) without transplantation of hematopoietic cells.

EXPERIMENTAL RESULTS

The effect of pertussis vaccine on hematopoietic cells remaining in the animal after irradiation on a sublethal dose, giving rise to endogenous colonies in the spleen (endogenous CFU-S) was studied in the experiments of series I. Experiments were carried out on mice of different genotypes and the vaccine was injected at different times before irradiation (Table 1). As will be clear from Table 1, pertussis vaccine had a comparable action on CFU-S of mice with a different genotype: The maximal stimulating effect for a dose of 10^{10} BC was observed when the vaccine was injected 1 day (C57BL/6 mice) or 3 days (BALB/c and CBA mice) before irradiation. The peak of the number of endocolonies in the (CBA × C57BL/6)F_1 hybrids occupied an intermediate position (1st-3rd days) compared with the response of the two parental strains. The stimulating action of a dose of 10^{10} BC was preserved even if injected into BALB/c, CBA, and F_1 hybrid mice 9 days before irradiation, whereas in C57BL/6 mice, the peak of whose response was shifted compared with other strains of mice, the stimulating effect was weak even when the vaccine was injected 5 days before irradiation ($P > 0.05$). A low dose of vaccine (10^7 BC) also had a stimulating action on endocolony formation, but its effect was "delayed" compared with the action of a dose of

TABLE 2. Number of CFU-S in Bone Marrow and Spleen of CBA Mice after Immunization with Pertussis Vaccine (M ± m)

Times of injection (before transplantation)	Dose of vaccine, BC	Bone marrow		Number of recipients	Spleen		Number of recipients
		number of CFU-S, 10^6 NC	number of cells per femur, $\times 10^6$		number of CFU-S, 10^6 NC	number of spleen cells, $\times 10^6$	
Control		304±14 (274—334)	18,8	37	27,8±1,3 (25,1—30,5)	162,5	36
1 day							
	10^7	320±14 (290—350)	17,9	23	26,4±1,4 (23,8—29,0)	135,0	36
	10^{10}	380±12* (356—404)	16,8	30	36,6±1,6* (33,4—39,8)	155,0	30
3 days							
	10^7	356±22 (312—400)	14,4	20	34,8±2,0* (32,0—38,6)	132,5	32
	10^{10}	440±14* (411—469)	24,0	30	48,2±2,0* (44,4—52,0)	171,7	34

Legend. At least 6 mice were used as donors in each group. Confidence interval, calculated at the P = 0.05 level, given in parentheses. NC) nucleated cells. Mean results of three experiments shown.
*Differences from control significant.

10^{10} BC and its intensity was weaker. The greatest stimulation of the number of endocolonies was observed when a low dose of vaccine was injected 3-9 days before irradiation. The number of endogenous foci of hematopoietic cells in the spleen of F_1 mice vaccinated with a dose of 10^7 BC did not differ significantly from the control at any time of observation. One cause of the increase in endocolony formation observed in mice in the early period after injection of pertussis vaccine may be intensified proliferation of hematopoietic stem cells in the bone marrow, and as a result of their increased migration into the spleen. To test this possibility, pertussis vaccine was injected into CBA mice 1 or 3 days before transplantation of the cells into lethally irradiated recipients (vaccination at these times had a maximal stimulating action on endogenous CFU-S) and the number of CFU-S was determined in the bone marrow and spleen of the vaccinated mice.

The results show (Table 2) a significant increase in the number of CFU-S in the bone marrow of mice vaccinated with a dose of 10^{10} BC 1 day and in particular, 3 days before transplantation (380 and 440 CFU-S per 10^6 bone marrow cells respectively compared with 304 CFU-S in the control). A similar increase both in the concentration of CFU-S in their absolute number was observed in the spleen of mice vaccinated with 10^{10} *B. pertussis* cells 1 day or 3 days before transfer. On immunization of mice with a dose of 10^7 BC 3 days before transplantation, an increase in the concentration of CFU-S in the spleen was discovered, but their absolute number was unchanged compared with the control: 4614 and 4517 CFU-S per spleen, respectively.

A stimulating action on endogenous CFU-S similar to the action of pertussis vaccine is exhibited by bacterial endotoxins [13], heterologous plasma [8], antilymphocytic serum [5], *Mycoplasma arthritidis* [1, 2], and other agents [6]. However, unlike *M. arthritidis* which had no stimulating action on endocolony formation in C57BL/6 mice [4], pertussis vaccine was effective when injected into mice of this strain. The action of a dose of 10^7 BC on endogenous CFU-S was complex in character and the stimulating effect was manifested in the time of injection of the vaccine, which was a longer time away from irradiation of the mice than when a dose of 10^{10} BC was used. The "delaying" character of the action of a small dose of vaccine may reflect the more gradual involvement of stem cells in proliferation or in migration and in compensatory proliferation, but these are matters for further study.

Investigation of the number of CFU-S in the bone marrow and spleen showed an increase in their number in these organs 1 and 3 days after vaccination of the mice in a dose of 10^{10} BC, in agreement with results obtained previously showing stimulation of recirculation of CFU-S after injection of killed pertussis vaccine [7, 11]. Infection of mice with *M. arthritidis*, which stimulates endocolony formation strongly [1, 2], was not accompanied, however, by an increase in the number of transplantable CFU-S in the bone marrow and spleen [3]. A similar result was obtained when the action on CFU-S of stimulators of endocolony formation such as endotoxin and heterologous plasma [8] or antilymphocytic serum [5] was studied.

These contradictions can be explained on the grounds that endogenous CFU-S are evidently part of a population of stem cells in a state of active proliferation [9], whereas the transplantable CFU-S are perhaps mainly in the G_0 stage or in the long G_1 phase [10]. There is evidence that sublethal irradiation synchronizes cells in the S phase to some degree, and in that phase CFU-S are more resistant to irradiation than cells not in the cycles [9, 15]. It can be postulated that pertussis vaccine and *M. arthritidis* differ in their effects on the proliferative state of the CFU-S population, and this problem is the subject of a current study.

LITERATURE CITED

1. D. R. Kaulen, A. V. Sanin, V. V. Khorobrykh, et al., Zh. Mikrobiol., No. 5, 72 (1980).
2. A. V. Sanin, D. R. Kaulen, V. V. Khorobrykh, et al., Byull. Éksp. Biol. Med., No. 8, 179 (1979).
3. A. V. Sanin, V. V. Khorobrykh, A. V. Pronin, et al., Nauch. Dokl. Vyssh. Shkoly, Biol. Nauki, No. 4, 95 (1980).
4. A. V. Sanin, I. V. Rakovskaya, and V. V. Khorobrykh, Mikrobiol. Zh., No. 2, 199 (1981).
5. I. L. Chertkov and L. N. Lemeneva, Probl. Gematol., No. 12, 44 (1971).
6. I. L. Chertkov and A. Ya. Fridenshtein, The Cellular Basis of Hematopoiesis [in Russian], Moscow (1977).
7. D. W. H. Barnes and J. F. Loutit, Nature, 213, 1142 (1967).
8. D. R. Boggs, J. C. Marsh, P. A. Chervenick, et al., J. Cell Physiol., 71, 227 (1968).
9. S. S. Boggs, D. R. Boggs, G. L. Neil, et al., J. Lab. Clin. Med., 82, 727 (1973).
10. S. S. Boggs and D. R. Boggs, J. Lab. Clin. Med., 82, 740 (1973).
11. F. C. Monette, S. S. Morse, et al., Cell Tissue Kinet., 5, 121 (1972).
12. K. Nooter and P. Bentvelzen, J. Natl. Cancer Inst., 57, 115 (1976).
13. W. W. Smith, G. Brecher, R. A. Budd, et al., Radiat. Res., 27, 369 (1966).
14. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).
15. J. E. Till and E. A. McCulloch, Radiat. Res., 18, 96 (1963).

ROLE OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE IN THE ACTION OF ESTRADIOL IN UTERINE TISSUE

N. N. Garnovskaya, I. L. Dumler, and R. N. Étingof

UDC 612.627.015.1.014.46:615.256.51

KEY WORDS: phosphodiesterase; estradiol; uterus.

Changes in the cyclic nucleotide concentration in the cells of animal tissues under the influence of hormones as a rule take place on account of changes in activity of adenylate cyclase, associated with receptors for hormones in the outer membrane of cells which react to them [3, 14]. As regards another enzyme involved in cyclic nucleotide metabolism, namely phosphodiesterase (PDE), concentrated mainly in the cell cytosol, according to the generally accepted view its role can be reduced to removal of an excess of cyclic nucleotides formed as a result of activation of adenylate cyclase, the immediate target for the action of a hormone entering the cell.

The sole and unique exception to this rule is the photoreceptor cell of the retina (rod). In this cell the receptor of photons (rhodopsin) is linked with PDE and, as a result of illumination, the activity of this enzyme changes sharply [1, 5, 6]. The nature of the stimulus reaching the cell in this particular case is unusual (quanta of light). In addition, this stimulus does not arise from the internal medium, like a hormone, but from the external

Laboratory of Biochemical Bases of Reception, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. G. Baranov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 6, pp. 110-112, June, 1982. Original article submitted September 24, 1981.