by the 3rd day and recovered only at the end of the month. The suppressor activity of the spleen cells fell toward the end of the 1st day after trauma and returned to normal at the beginning of the 3rd week after burning.

The results thus indicate that activity of B lymphocytes and T helpers increases in mice after burns affecting 10% of the body surface, but activity of T suppressors declines. After an extensive burn functional insufficiency of the immunoregulatory T lymphocytes develops, whereas the B cells retain their power to form antibodies against SRBC. It can accordingly be concluded that the development of immunodepression in severe burns is connected with injury to the T system of lymphocytes.

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SUPPRESSIVE ACTION OF XENOGENEIC BONE MARROW CELLS ON ANTIBODY FORMATION IN SPLEEN CELL CULTURES in vitro

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KEY WORDS: bone marrow suppressor; immunodepression.

Recent investigations have demonstrated the suppressive action of cells of bone marrow origin on the immune response [1, 7, 9]. The addition of syngeneic bone marrow cells to cultures of spleen cells depresses the primary response to sheep's red blood cells (SRBC) practically completely [2-5, 10]. It has been suggested that bone marrow suppressor cells "prohibit" development of the immune response on the territory of the bone marrow [3]. The role and mechanisms of action of these regulatory cells has not been finally explained. It has been shown in vitro that suppression of the primary immune response to SRBC can be effected by allogeneic bone marrow cells [5].

The object of this investigation was to study the effect of xenogeneic bone marrow cells on induction of the primary immune response in cultures of mouse spleen cells in vitro.

EXPERIMENTAL METHOD

(CBA $\times$ C57BL)F_1 mice aged 2-4 months were used. Noninbred piglets aged 3 months, White Leghorn hens aged 4 months, guinea pigs aged 2 months, and Wistar rats aged 3 months served as donors of bone marrow cells. Induction of the primary immune response in cultures of spleen cells to SRBC was carried out by a modified Click's method. Spleen and bone marrow cells were cultured in the ratio of 1:1 in "GIBCO Serumless medium" in concentrations of 2.5×10^6 and 5×10^6 cells/ml. Additional substances added included fetal serum (10%), glutamine (200 mM), and 2-mercaptoethanol (10^{-5}M). The viability of the cells was determined by staining with trypan blue and eosin. SRBC kept in Alsever's solution served as the antigen. The number of 19S antibody-forming cells (AFC) was counted after culture for 4 days in vitro by Jerne's method [8]. The experimental data was subjected to statistical analysis.

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TABLE 1. Effect of Xenogeneic Bone Marrow Cells on AFC Production in Cultures of (CBA × C57BL)F_1 Mouse Spleen Cells in Vitro

Original number of cells in culture (× 10^6/ml)						Survival rate of cells at end of incubation, %	Number of AFC after culture for 4 days (× 10^6 living cells)	Suppression of antibody formation, %
mouse spleen cells	(CBA × C57BL)F_1 mouse BM	guinea pig BM	pig BM	rat BM	hen BM			
2,5	—	—	—	—	—	47	651,30±133,65	—
5	—	—	—	—	—	38	661,18±118,68	—
2,5	2,5	—	—	—	—	46	30,60±9,75	96
2,5	—	2,5	—	—	—	37	5,10±2,68	99,9
2,5	—	—	—	—	—	44	876,00±98,23	—
5	—	—	—	—	—	72	738,66±127,36	—
2,5	2,5	—	—	—	—	46	82,00±36,67	91
2,5	—	—	2,5	—	—	49	0	100
2,5	—	—	—	—	—	29	572,46±145,38	—
5	—	—	—	—	—	39	402,43±43,00	—
2,5	2,5	—	—	—	—	42	23,76±6,87	96
2,5	—	—	—	2,5	—	26	47,23±15,26	92
2,5	—	—	—	—	—	36	178,00±59,2	—
5	—	—	—	—	—	54	178,00±40,9	—
2,5	2,5	—	—	—	—	39	16,00±7,2	91
2,5	—	—	—	—	2,5	20	7,00±8,0	97

Legend. BM) Bone marrow; each experiment was repeated twice or three times.

EXPERIMENTAL RESULTS

The data in Table 1 show that addition of syngeneic bone marrow cells to mouse spleen cultures led to the virtually total suppression of the primary immune response to SRBC. Accumulation of AFC under these circumstances was reduced by 91-96%, in agreement with data in the literature [2, 3]. The addition of xenogeneic bone marrow from animals of different species (guinea pigs, pigs, rats, hens) to the spleen cell cultures was followed by the same suppression of AFC accumulation as when syngeneic bone marrow was added. Suppression of AFC production by bone marrow cells, incidentally, had practically no effect on the survival rate of the cells in culture.

The results thus indicate that activity of bone marrow cells limiting the development of the immune response takes place even in the presence of a xenogeneic barrier. A previous experimental analysis of bone marrow suppressors of syngeneic antibody producers showed that the effector cells determining the immunosuppressive activity of the bone marrow are cells of the B lymphocyte series [5]. The same investigation showed that the suppression effect is accompanied by total inhibition of proliferative activity of the spleen cells.

The results obtained, showing that the mechanism of the suppressive effect of bone marrow is independent of the immunologic compatibility of the partner cells, are yet another argument in support of the view that any proliferative cell can be targets for bone-marrow suppressors. Experiments using a two-chamber culture system have shown that bone marrow suppressor cells produce a humoral factor which can pass through a nucleopore membrane [3]. The immunosuppressive action of this factor is effected through the blocking of proliferation of lymphoid cells.

The possibility cannot be ruled out that xenogeneic spleen or lymph node cells may have a suppressive action on antibody production in vitro. Suppressor cells capable of inhibiting antibody production in vitro by spleen cells of young mice in response to antigens of heterologous erythrocytes are known to appear in the spleens of old mice [11]. Corvalan and Howard [6] showed that rat spleen cells cannot respond in vitro to SRBC because of the presence of suppressor cells in the spleen. Accordingly, the question of whether the property of suppression of antibody production is peculiar to cells of xenogeneic bone marrow or whether other xenogeneic cells possess this quality cannot yet be regarded as finally settled. The data obtained in the present investigation are evidence of a suppressive action of bone marrow on antibody production in culture and in a xenogeneic system.

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BLAST TRANSFORMATION IN RESPONSE TO PLANT MITOGENS IN WISTAR RATS INFECTED WITH *Mycoplasma arthritidis* OR *Acholeplasma laidlawii*

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The action of mycoplasmas on the lymphoid system is known to depend largely on the infecting dose and on ability to utilize arginine [5]. If high concentrations of arginine-utilizing mycoplasmas are added in vitro, marked suppression of the response to plant mitogens is observed and is due to removal of arginine from the culture medium [10]. If the multiplicity of infection is reduced, the inhibitory effect is replaced by a stimulant effect [5]. Mycoplasmas utilizing dextrose in most cases have only a stimulant action regardless of the dose [3, 7, 9]. The results of investigation of blast transformation of lymphocytes taken from infected animals are highly contradictory [3]. In some cases suppression of the response to mitogens after infection with species of arginine-utilizing mycoplasmas could not be found [6], but in other cases it was observed [8]. Infection of animals with dextrose-utilizing mycoplasmas does not affect blast transformation due to plant mitogens [3].

The object of the present investigation was to study the action of phytohemagglutinin (PHA) and concanavalin A (con A) on lymphocytes of rats infected with *Mycoplasma arthritidis* and *Acholeplasma laidlawii*.

EXPERIMENTAL METHOD

The animals were infected with mycoplasmas as described previously [2]. Intact rats and rats into which broth was injected served as the control. The proliferative activity of spleen cells and mesenteric lymph node cells was estimated 7, 14, 28, 33, and 65 days after the beginning of infection. The blast transformation reaction was set up in 3040 Microdisks (Falcon Plastics). Into each well 5×10^5 cells were introduced in a final volume of 0.2 ml medium. The culture medium was RPMI-1640 medium with 10% normal rat serum, 1% 1 M HEPES solution, 1% L-glutamine, 100 units/ml benzylpenicillin, and 100 μg/ml streptomycin-calcium chloride complex. The Microdisks were incubated at 37°C in a humid atmosphere with 5-7% CO_2. Preliminary experiments showed that the optimal concentration of PHA (PHA-P, from Difco) was 0.5 μg/ml and that of con A (from Sigma) was 10 μg/ml. The reaction was read on the 4th day of culture. DNA synthesis was determined by measuring incorporation of thymidine-^{3}H (2.5 μCi/ml, 1 Ci/mmole), added 16 h before the end of the experiment, by the method described previously [1].

EXPERIMENTAL RESULTS

It will be clear from Figs. 1 and 2a that injection of broth or of *A. laidlawii* in vivo caused no appreciable changes in the intensity of thymidine-^{3}H incorporation by the spleen and lymph node cells at all times of the

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