Discussion. In experiments in which human serum and polymorphonuclear leukocytes are examined for their phagocytic or bactericidal activity, combinations of cells and serum of the same blood type, of O cells with any other type of serum, or of AB serum or pooled serum of nonspecified blood type with cells of any type, are in general use. So far only a few articles have dealt specifically with the problem of the effects of ABO blood types on phagocytosis. With combinations of leukocyte-rich buffy coats and *Staphylococcus aureus,* Tandon[5] examined the phagocytic activity of leukocytes of different blood types and found that O cells have the highest activity, followed by A, B and AB. He speculated that blood group substances in the leukocytes or isohemagglutinins may affect the opsonization of bacteria, although no substantial evidence was offered. When the phagocytic activity of leukocytes was compared in compatible and incompatible sera, Melby[6] noted that O cells are the least and AB cells the most active and that the use of incompatible serum, mostly O serum, generally leads to a reduced uptake of *E. coli.* Therefore, he emphasized that full compatibility is essential in the study of phagocytosis. One of the observations of Melby relevant to the present study is that heat-treatment of serum abolishes the difference, i.e., the serum factors responsible for the reduction of phagocytosis are in the heat-labile fraction. As noted above, heat lability is a feature of opsonin activity against zymosan. Although it remains to be determined whether or not heatstable factors contribute to the opsonization of zymosan, these independent observations indicate that the removal of presumably heat-stable isohemagglutinins does not affect the opsonin activity of O serum. The present experiment shows that a higher dilution of O serum causes a more pronounced decrease in the capacity to opsonize zymosan than dilutions of any other type of serum. This defect appears to reside in the serum, as O cells can consume oxygen equally well when combined with A, B, or AB serum. This decrease of opsonin activity occurred only against zymosan, and contrary to previous reports, bacterial species were not influenced at all by differences in ABO type. The use of live or killed bacteria and their greater response to antibodies as opsonins may explain the difference, but it is not yet completely understood. Such high dilutions of serum are usually not used for opsonization, so the defect revealed in this experiment may not have any practical significance. However, this finding should be considered when kinetic studies of opsonization are conducted with zymosan, and possibly with other particles, in various combinations of sera and cells.

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Effect of azimexon (BM 12.531) on mouse granulocyte-macrophage and monocyte-macrophage progenitor cells[1]

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Summary. Treatment of mice with 25 mg/kg azimexon (BM 12.531) resulted in an increase in granulocyte-macrophage colony-forming cells (GM-CFC) in spleen and bone marrow after a transient depression in the cell populations. Bone marrow monocyte-macrophage colony-forming cells (MM-CFC) increased at 7 days after treatment, and splenic MM-CFC were least affected by azimexon treatment. The increase in granulocytic and monocytic colony-forming cells may play a role in the previously reported protection by azimexon against radiation and drug-induced toxicity.

A series of cyanoaziridine compounds have been studied recently for their immunomodulatory properties[2-7]. Azimexon (BM 12.531, chemical name 2-[2-cyanaziridinyl-(1)]-2-[-2-carbamoylaziridinyl-(1)]-propane) has shown promising results in a number of immunological evaluations. Treatment of mice with azimexon resulted in an increase of delayed-type hypersensitivity reactions[2,3], improved resistance to infections[4,5], activation of macrophages in vivo and in vitro[2,3], augmentation of natural killer cells[6], and prolongation of mean survival time of tumored mice[3]. Addition of azimexon to human lymphocyte cultures in vitro resulted in an enhancement of their blastogenesis in response to mitogens[7], whereas a dose-dependent activation of T lymphocytes from patients treated with azimexon has also been reported[2]. Azimexon treatment increased the survival of mice given cyclophosphamide[8], and the survival of mice receiving total-body X-irradiation was increased when the drug was administered either before[3] of after[8] irradiation. There is evidence that azimexon helps reverse the leukopenia resulting from irradiation[8], cyclophosphamide treatment[4], or combined cytoreductive chemotherapy[9], which suggests a useful adjuvant role of this drug in cancer therapies or in treatment of radiation accident victims.

One of the many aspects of stimulation of the immune system by biological response modifiers is their effects on stem and progenitor cells. The hematopoietic stem cell, CFU-s, is the ultimate precursor of erythrocyte, granulocyte, macrophage, megakaryocyte, and eosinophil differentiated progeny[10]. Two progeny of the CFU-s, the bone marrow MM-CFC[11] and the GM-CFC[12], are of special interest when considering the reticuloendothelial system, since they are early or primitive cells in the sequence from stem cell to mature end cell. The GM-CFC is committed to the granulocyte and mononuclear phagocyte pathway at differentiation, and the MM-CFC is committed exclusively to the mononuclear phagocyte pathway. In considering possible mechanisms for the immunoprotection shown by

azimexon, we studied its effects on the in vitro colony-forming capability of the GM-CFC and MM-CFC.

Materials and methods. Azimexon, obtained from Boehringer Mannheim GmbH, Mannheim (FRG), was dissolved in physiological saline and sterile filtered before injection. Female B6D2F1 mice, 2-4 months of age, were injected i.p. with azimexon (25 mg/kg b.wt) or with an equal volume of saline for control experiments. Sterile cell suspensions from spleen and femoral bone marrow were prepared in 10% fetal calf serum-supplemented, phosphate-buffered saline (Flow Laboratories, McLean, Virginia), and the cells were separated by repeated passage through a 20-gauge hypodermic needle. Cells from 2 or 3 animals were pooled, and nucleated cell counts were recorded for overall organ cellularity. In vitro culture was according to a modification[13] of the double agar culture technique first described by Bradley et al.[14]. Pregnant mouse uterine extract was used as the source of colony-stimulating activity. Cultures were plated in concentrations of 1×10^5 splenic and 2×10^4 bone marrow cells per 35-mm petri dish. Incubation was done at 37 °C in 10% CO_2. Colonies (clusters of ≥ 50 cells) counted at 7 days after the initial plating were considered to be derived from GM-CFC cells. Those colonies arising after 25 days of incubation were scored as MM-CFC progeny. Mann-Whitney nonparametric statistics were used to determine the significance of differences between colonies per organ at different days after azimexon treatment.

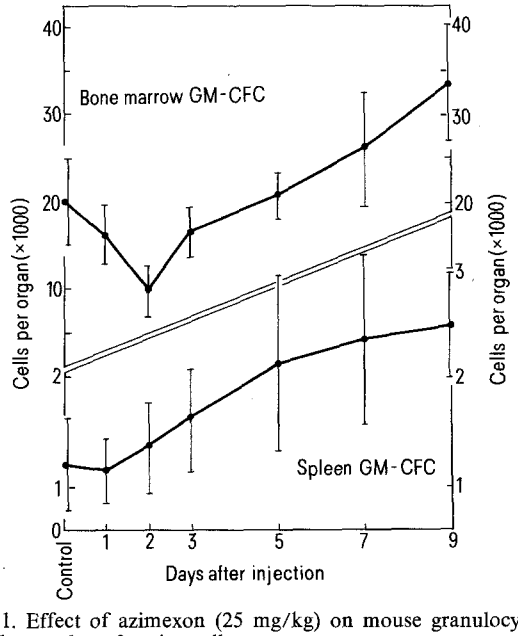

Figure 1. Effect of azimexon (25 mg/kg) on mouse granulocyte-macrophage colony forming cells.

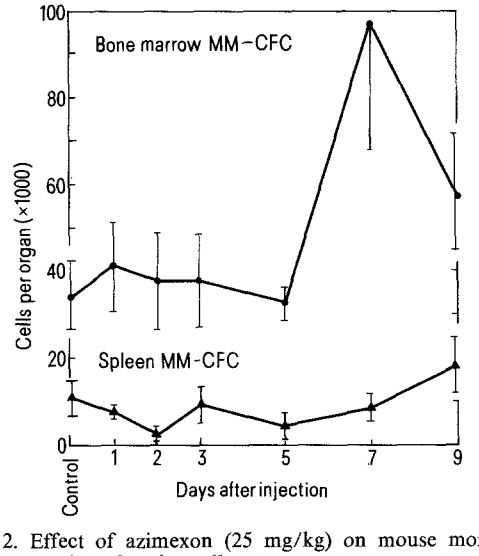

Figure 2. Effect of azimexon (25 mg/kg) on mouse monocyte-macrophage colony forming cells.

Results and discussion. The total cellularity of marrow and spleen were determined on various days after treatment with azimexon (25 mg/kg b.wt). The mean cellularity of bone marrow (2.25×10^8) or spleen (3.25×10^8) did not differ significantly after treatment with this drug. Indeed, inherent cytotoxicity of azimexon must be low, for preliminary studies done with 250 mg/kg b.wt azimexon demonstrate recovery of cell populations to control values within 3.5 days after treatment (unpublished data).

In both bone marrow and spleen, azimexon administration resulted in an initial slight depression in the number of GM-CFC, followed by a steady increase in population size over the balance of the 9 days (fig. 1). Splenic GM-CFC population on the days of maximum stimulation (days 7 and 9) was significantly higher than that of the saline control ($p < 0.05$) as well as days 1 and 2 ($p < 0.05$). Bone marrow GM-CFC populations on days 7 and 9, although not significantly higher than that of the saline control, were significantly higher than days 1 and 2 ($p < 0.01$). Azimexon had little effect on bone marrow MM-CFC for the first 5 days, followed by a steep rise over days 7 and 9 (fig. 2). This elevation was significantly higher than the MM-CFC control ($p < 0.001$) and days 1 and 2 ($p < 0.05$). Splenic MM-CFC cells were influenced the least by azimexon (fig. 2). The shallow depression present at days 1 or 2 is followed by a weak upward trend for the balance of the 9 days. Days 7 and 9 do show a marginally significant increase over the initial depression ($p < 0.05$).

The lack of splenic MM-CFC response to azimexon may be due to the fact that the bone marrow MM-CFC, apparently, is heterogeneous whereas the splenic MM-CFC population may be homogeneous. Sedimentation velocity studies[13] indicate that a slow sedimenting MM-CFC is found in both bone marrow and spleen and a faster sedimenting MM-CFC is found also in the bone marrow. It is interesting to note that the bone marrow MM-CFC demonstrated a greater stimulation by azimexon treatment, and may indicate that only one of the 2 described MM-CFC subpopulations is sensitive to azimexon. The kinetics of the stimulatory effects of azimexon on colony-forming cells appears to be different when compared to other biological response modifiers[15-20]. Whereas *C. parvum*[15,16], *Bacillus Calmette-Guérin*[19], glucan[19]. and pyran[20] all begin to stimulate hematopoietic CFC within 24 h after treatment, azimexon demonstrates a lag time. This is most pronounced in bone marrow MM-CFC, where stimulation occurs only after 5 days from the time of initial treatment.

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The control of the frog *(Rana esculenta)* thumb pad[1]

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Summary. Extirpation of the pars distalis of the pituitary or castration in any period of the year cause thumb pad regression. Thumb pad development is regulated by an interaction of both temperature and androgenic hormones.

The thumb pad in male anurans represents an androgen-dependent secondary sexual characteristic[2]. Histological observations (height of the epidermis and glandular epithelium and mitotic rate of the epidermal cells) and biochemical studies (DNA, RNA and protein content and β-glucuronidase activity) have demonstrated that the thumb pad of the green frog, *Rana esculenta*, undergoes regression following hypophysectomy or castration; androgen therapy stimulates its development[3-7]. Its seasonal developmental cycle (regression in summer and development in autumn and early winter) has been correlated with annual cyclic fluctuations in the Leydig cell-secretory activity and plasma androgen levels[8-10]. In addition to this, Rastogi et al.[11] showed that high temperature (28 °C) cause thumb pad regression and low temperature (4 °C) tend to maintain, if not to stimulate, its integrity. Furthermore, in frogs housed at 28 °C exogenous testosterone does not stimulate the thumb pad. These facts led us to undertake a series of experiments in order to analyze better the interaction of hormonal and environmental factors in the control of thumb pad development.

Materials and methods. Experimental series 1: Pars distalis of the pituitary was removed in animals caught in February, June, August and October, as described earlier[12]. Animals were sacrificed at different time intervals. Pars distalis homogenate, in deionized water, was injected into the dorsal sac of operated frogs. Each treated frog received a total of 2 partes distales given in 6 equal fractions, each 3rd day.

Experimental series 2: Group A (January-April 1971). 75 days after castration the animals were treated with different doses of testosterone propionate (TP); group B (September-October, 1979). 28 days after castration the animals were treated separately with TP or dihydrotestosterone (DHT) at either 20 °C or 4 °C; group C (September-October, 1980). 30 days after castration all frogs were kept at 4 °C and treated separately with TP or DHT; group D (January-March, 1981). 28 days after castration the animals were treated separately with TP or DHT at 8 °C and 16 °C. The total dose mentioned here was given in 6 equal fractions. The i.m. injections (group A) were all in 0.1 ml amond oil, whereas the s.c. injections, in the dorsal sac,

Table 1. Influence of the removal of the pars distalis and homologous pars distalis homogenate on thumb pad development*

Treatments**	Thumb pad epidermis	Glandular epithelium
February initial control	100±9 (well developed)	100±13
PDX 50 days	60±5***	58±2***
PDX 90 days	30±1***	37±2***
PDX 90+PD	93±6	98±4
June initial control	100±12 (regressed)	100±7
PDX 40 days	89±8	92±5
PDX 100 days	60±9***	68±6***
PDX 100+PD	146±6***	137±6***
August initial control	100±8 (markedly regressed)	100±11
PDX 50 days	96±5	91±8
PDX 150 days	87±7	90±6
PDX 150+PD	164±11***	156±10***
October initial control	100±9 (developed)	100±10
PDX 40 days	71±3***	69±7***
PDX 100 days	47±2***	38±14***
PDX 100+PD	179±9***	162±15***

* Each control value is represented by 100 and that for treated groups is expressed as a percentage of the equivalent control value. ** All experimental animals were maintained at room temperature (in summer 18-22 °C; in winter 12-18 °C). PDX, pars distalis removed; PD, pars distalis homogenate. *** Significance of difference vs equivalent control $0.01 < p < 0.02$.