Mouse Serum Factor Depressing Lymphocyte Transformation

In a variety of human diseases as diverse as alcoholic cirrhosis of the liver[1] and lepromatous leprosy[2] serum or plasma factors occur which depress mitogen-induced lymphocyte transformation in vitro (for list of these diseases see ref.[1] and[2]). We[2] and others[3] have suggested that the factors responsible might not be new disease-specific factors but might represent over-production of factors which normally operate to control the development and/or expression of cell-mediated immunity. In the course of attempts to produce an animal model on which to test this hypothesis we found that sera from normal mice contained a factor which depressed lymphocyte transformation in vitro.

The mice used were of the following strains: A/J and CBA/J (The Jackson Laboratory); the F1 cross between these strains; non-inbred SW (University of Sydney Animal Farm); and Nude mice (C.S.I.R.O. Division of Animal Genetics, Sydney). Spleen cells were cultured with phytohaemagglutinin (PHA) or in mixed lymphocyte reactions by a modification of the technique of ADLER et al.[4] (see footnote to Table I). The extent of lymphocyte transformation was determined by measuring the incorporation of tritiated thymidine in the last 24 h of incubation. The optimum dose of PHA and the time at which thymidine incorporation was maximal were determined for each strain of mice. For mixed lymphocyte reactions A/J and F1 spleen cells were mixed in equal numbers; thymidine incorporation was maximal after 4 days. Serum from 3 to 12 mice were pooled, sterilized by Millipore filtration and stored at –20°C. PHA provides an immunologically non-specific stimulus to thymus-derived (T) lymphocytes[5,6] and the cells responding specifically to allogeneic cells are T-lymphocytes[6].

In a series of experiments mouse spleen cells were cultured in a medium containing 2.5% human serum; 5, 2.5, 1.25% or no mouse serum; and PHA at the optimal dose or $^1/_4$ thereof. A/J and F1 spleen cells and mixtures of the two were cultured in similar media without PHA. Table I shows that 5% mouse serum depressed the responses of cells to $^1/_4$ the optimal dose of PHA and to allogeneic cells. Other experiments showed that the degree of depression was directly dependent on the concentration of mouse serum; that it was less marked when an optimal dose of PHA was used; and that the same concentration of mouse serum was less depressive in higher than in lower concentrations of human serum.

The depressive factor in normal SW mouse serum has been partially characterized. It was not affected by heating to 56°C for 20 min. It was not removed by dialysis; in fact some batches of sera become more depressive on dialysis against RPMI 1640. It was present in plasma from mice previously injected with heparin and is thus not a product of clotting. When SW serum was fractionated on DEAE-cellulose, with stepwise elution by phosphate buffers, pH 7.8, from 0.01 *M* to 0.2 *M*, no activity was recovered. However, elution with 0.2 *M* buffer plus 2 *M* NaCl resulted in complete recovery of inhibitory activity. Gel filtration on Sephadex G-200 revealed all the depressive capacity of SW serum in the third peak. Likewise, the depressive capacity of F1 serum

Table I. Inhibitory effect of normal mouse serum on lymphocyte transformation[a]

Spleen cells	Mouse serum Strain	Concentration (%)	PHA (µl)	cpm per 10^6 cells	Inhibition (%)
SW	SW[b]	0	0.06	8168	
		5	0.06	3147	61
CBA	CBA	0	0.125	2427	
		5	0.125	419	83
A	A	0	0.125	6822	
		5	0.125	1616	76
F1	F1	0	0.125	12702	
		5	0.125	3337	74
A + F1	F1	0	0	1992	
		5	0	619	68

[a] The cultures were set up in sterile 2.2 ml plastic vials (Stayne Laboratories, Code E2/R). The reaction mixtures contained 0.6 ml spleen cell suspension (7.5×10^6 cells/ml), 0.2 ml 12.5% heated (56°C, 15 min) human serum, 0.2 ml 25% mouse serum or RPMI 1640, and 0.1 ml diluted PHA. The volume of the original bottle of PHA-P (Difco, Control 550408) was 5 ml and the quantity of PHA added was calculated on this basis. Thus 0.125 µl was contained in 0.1 ml of a 1:800 dilution. 24 h before harvest each culture received 0.2 ml RPMI 1640 containing 2 µCi tritiated thymidine (The Radiochemical Centre, TRA. 120, 5.0 Ci/mmol). The cultures were harvested as described elsewhere[2] and counted in a Philips LSA 400 liquid scintillation counter. Results are means of duplicates, calculated as cpm per 10^6 cells after correction for background. Over a large number of experiments the average deviation of individual cultures from the mean of the duplicates was ± 5%.

[b] 3 pools of SW serum similarly tested with $^1/_4$ the optimal dose of PHA showed a mean inhibition of 56% (S.E. ± 6%). 14 pools tested with an optimal dose of PHA (0.25 µl) showed a mean inhibition of 34% (S.E. ± 4%).

Table II. Effect of serum from BCG-treated mice on lymphocyte transformation

Time after 2nd BCG injection	Tritiated thymidine incorporation as % of mean incorporation in normal control sera, mean and range	
	Normal	BCG-injected
14 days	100 (92–108)	59 (46–67)
56 days	100 (73–110)	87 (82–95)

SW mice were injected i.p. with 0.5 mg live BCG vaccine (Commonwealth Serum Laboratories, Melbourne) on 2 occasions 6 weeks apart. They were bled 14 days and 56 days after the 2nd injection. Uninjected SW mice were bled at the same time. In each case 3 pools of sera were prepared, each made from 3 mice.

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for both PHA-induced transformation and mixed lymphocyte reactivity was found almost entirely in the third peak of a G-200 eluate.

It is of particular interest to determine whether the inhibitory capacity of mouse serum varies with changes in the immunological status of the donors. Sera were obtained from SW mice injected with live BCG vaccine, which causes proliferation of macrophages[7]. The effects of sera obtained after a single injection were variable, but the depressive effect was generally not greater than that of normal sera. As shown in Table II, sera obtained from mice 2 weeks after the second of 2 injections of BCG were substantially more depressive than normal sera. This difference was scarcely apparent 6 weeks later.

Sera were also obtained from SW mice treated with antilymphocyte globulin (ALG) which causes, among a variety of biological effects[8], a temporary depletion of T lymphocytes[9] and may cause some proliferation of macrophages[10]. They received 3 s.c. injections, at daily intervals, of 0.5 mg of a preparation shown to be immunosuppressive[11]. The sera had an increased depressive capacity up to 6 days after the first injection (mean thymidine incorporation 56% of that in normal mouse serum). A second wave of increase in depressive capacity occurred later, maximal at 18 days (mean thymidine incorporation 35% of that in normal mouse serum).

Nude mice (genetically *nu/nu*) lack T lymphocytes[12] and cannot make cell-mediated immune response[13]. Sera from such mice were compared with sera from heterozygous (*nu/+*) siblings, at concentrations from 1.25 to 5% in the presence of 2.5% human serum and optimal or $^1/_4$ optimal doses of PHA with spleen cells from *nu/+* mice. Both sets of sera had a pronounced depressive capacity (67% for *nu/nu* serum and 63% for *nu/+* serum at 5% and with an optimal dose of PHA).

These findings indicate that normal mouse serum contains a factor which depresses the response of normal mouse spleen lymphocytes to PHA and to allogeneic lymphocytes. The factor appears to be a highly charged molecule of relatively low molecular weight. These properties make it highly unlikely that the factor is endotoxin[14] or an immunoglobulin. It is thus also unlikely that antibodies to PHA are responsible for inhibition of the response to PHA[15] and in any case mixed lymphocyte reactivity is also inhibited. The presence of depressive activity in sera from Nude mice and ALG-treated mice makes an origin from T lymphocytes very unlikely. Its possible origin from, e.g., bone-marrow derived (B) lymphocytes or macrophages is being investigated. Other current investigations concern the depressive effects of sera from mice subjected to more violent immunological insults, such as graft-versus-host reactions, and the effects of mouse sera on other activities of stimulated lymphcoytes, e.g., RNA and protein synthesis and cytotoxicity. The should shed light on the possible homeostatic role of the depressive factor and its relationship to the 'immunoregulatory α-globulin' isolated from some batches of normal human plasma[16] and the depressive factor(s) in normal chicken serum[17,18].

Résumé. Le sérum des souris normales contient un facteur, de haute charge négative et de faible poids moléculaire, qui abaisse la réponse de lymphocytes spléniques au PHA et aux lymphocytes allogéniques. Le sérum des souris qui ont eu une injection de BCG est même plus dépressif que le sérum des souris normales.

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Lymphocyte-Fibroblast Interaction in the Pathogenesis of Inflammatory Gingival Disease

Gingivitis is a destructive inflammatory disease of the tissues surrounding the teeth. It is extremely widely spread in human populations[1]. The disease is related etiologically to the accumulation and growth of microorganisms (specifically termed dental plaque) on the surface of the teeth[2]. The early gingival lesion is characterized by an infiltration of leucocytes and a concomitant reduction in the amounts of connective tissue substance. While the disease has been the object of intensive investigation, the nature of its pathogenesis has not been defined.

The data reported here were derived from an extensive series of studies using quantitative morphometric techniques for the analysis of human gingival biopsy specimens. The techniques used for preparation and analysis of the material are described in detail elsewhere[3] and a detailed report of the observations is being prepared[4]. The data show that a form of delayed hypersensitivity in which sensitized lymphoid cells appear to exert a cytotoxic effect on fibroblasts of the gingiva may play a major role in the induction and progress of the disease.

Volumetric data were obtained for the cell and fiber composition of the infiltrated connective tissue (ICT) and non-infiltrated connective tissue (NCT) of 20 human

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