Mitosis in liver macrophages after challenge with antigen

Challenge	Immunization	Mitoses per 100 fields (mean and range; 4 mice per group)
HSA	HSA in adjuvant	16 (10–24)
Saline	HSA in adjuvant	6 (2– 7)
HSA	None	2 (1– 5)
Saline	None	3 (2– 6)

counts of the numbers of hepatic parenchymal cell nuclei and Kupffer cell nuclei were made on representative sections. There were approximately 2700 parenchymal cell nuclei and 700 Kupffer cell nuclei per 100 fields. Comparison of sections from control and experimental mice revealed no differences of more than 5%.

The results are shown in the Table. Immunized mice challenged with antigen had 4 to 8 times as many mitoses as control mice and the range was completely outside the ranges for control mice. There was essentially no difference among the three control groups in the numbers of mitoses seen. In an additional experiment it was found that prolonging the vinblastine exposure for a further two hours increased the number of mitoses in antigen-challenged, immunized mice to 39 per 100 fields.

These findings indicate either that the Kupffer cells of appropriately immunized mice can, like free-floating peritoneal macrophages, be induced to divide when the animals are reexposed to a pure soluble antigen, or that the liver is a site where macrophage percursors which have entered the mitotic cycle come to ground. Although it is difficult to exclude the latter possibility, we favour the interpretation that the 'fixed' macrophages undergo mitosis, for the following reasons. The macrophages which undergo mitosis in the liver during the acquisition of cellular resistance to infection have been shown, by their prior ingestion of colloidal carbon, to have been previously resident in the liver[8]. Likewise, it has been shown, by the use of irradiation and appropriate shielding, that indigenous Kupffer cells proliferate in response to oestradiol[12]. Finally, there is a lag of 16 h between the injection of antigen into immunized mice and the onset of DNA synthesis in peritoneal macrophages[10], and the generation time of monocyte precursors in bone marrow has been estimated as 20 h[13]. If these figures are applicable to all cells of the mononuclear phagocytic system, it would seem unlikely that all, or even a majority, of Kupffer cells entering metaphase between 20 and 23 h after challenge could have been recently derived from an extra-hepatic site.

Our experiments shed no direct light on the mechanism of induction of mitosis but there is suggestive evidence, summarized above, that antigen-induced mitosis in macrophages is an expression of cell-mediated immunity. It has been reported that supernatant fluids from mixed cultures of allogenic rabbit lymphocytes can stimulate rabbit monocytes to proliferate in vitro and acquire some resistance to intracellular mycobacteria[14]. There is also evidence that macrophages can be activated by lymphokines produced by lymphocytes, derived from immune animals, after culture with specific antigen[15–18]. The relationship between activation and mitosis in mature macrophages is not clear[10,19]. It may be that the two phenomena are related but potentially independent expressions of cell-mediated immunity. It is also conceivable that mitosis in mature, fixed macrophages reflects a residual capacity to respond to the same stimulus which leads to proliferation of macrophage precursors in bone marrow and to the production of large numbers of new monocytes during the acquisition of cellular resistance to infection[20].

Résumé. Des souris ont été immunisées avec l'albumine du sérum humain dans l'adjuvant complet de Freund. Un jour après une injection de rappel de l'antigène, la taille des mitoses, parmi les macrophages du foie dépasse celle des mitoses des souris témoins.

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Specific Enhancement of the Cytotoxic Titre with Regard to Complement Source and Cytotoxicity Test Systems

In histocompatibility testing, there are still many problems associated with weak reactivity of the lymphocytes (CYNAP). It has been shown that the reactivity of human lymphocytes in cytotoxicity tests can be increased by pretreatment with enzymes. Whereas trypsin and ficin treatment may produce false positive results[1,2], neuraminidase gave an increased specific reactivity with human lymphocytes[3]. At the time of this investigation, studies with enzyme treated mice lymphocytes were not avaliable. Cytotoxicity tests are usually performed with rabbit serum as complement source. The above mentioned authors also employed this

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Table I. Reciprocal cytotoxic titres (> 50% dead cells) of anti C57 DBA serum in cytotoxicity assays with different complement and C57 lymphocyte preparations

One step; pretreatment with				Two step; no pretreatment				
None	Trypsin 0.25 mg/10^6	Ficin 0.025 mg/10^6	Neuraminidase 0.015–0.025 u/10^6	A	B	C	D	Absorbed complement
32	64	64	2.048	32	32	32	32	rabbit
4	16	16	4	4	8	16	32	guinea-pig, undiluted
0	0	0	0	4	8	16	32	guinea-pig, 1:10 diluted

Complement titres: rabbit serum 19.0 CH_{50}/ml; guinea-pig serum 190 CH_{50}/ml. A: supernatant removed; B: 1 × washed; C: 2 × washed; D: 3 × washed.

complement without considering guinea-pig serum as possible complement source. The purpose of this paper is to investigate the increased reactivity of enzyme treated mice lymphocytes with regard to the complement source, anticomplementarity of the serum, and the optimal cytotoxicity test system.

An experimental model with mice lymphocytes was chosen because in this system a marked anticomplementarity of mouse serum against guinea-pig complement is known[4] with at least two factors directed against bound C1 and C3 (unpublished data). By using this system, the effect of enzyme treated target cells on anticomplementarity of the serum can be studied, since MITTAL[2] suggested that anticomplementary effects may be reduced by enzyme (ficin) treatment. We used C 57 mice lymphocytes, anti C 57 DBA isoimmunserum, and both guinea-pig and rabbit serum as complement sources. Guinea-pig complement was employed undiluted and in a 1:10 dilution, which is equivalent to the complement activity of rabbit serum. Rabbit serum was absorbed twice on C 57 lymphocytes. The one step cytotoxicity test was performed essentially as described[5]. C 57 lymphocytes were obtained from spleen by sieving the minced organ through gauze with subsequent removal of red cells by differential lysis and 2 washings in Hank's solution. Lymphocytes were treated with enzymes by 15 min incubation at 37°C followed by 2 washings in Hank's. Antiserum was diluted with normal DBA serum and neuraminidase with saline containing 0.005 M $CaCl_2$. In the two step procedure, lymphocytes were first incubated with antiserum for 30 min at 37°C. After centrigufation at 400 g (4°C) and removal of the supernatant, sensitized lymphocytes were washed with Hank's before complement was added. Titres were read 15 min after staining with trypan blue.

In the one step procedure a specific titre increase from 1:32 to 1:2048 by treatment of mouse lymphocytes with neuraminidase can only be achieved with rabbit serum as complement source. Trypsin and ficin only give a slight titre raise with both rabbit and guinea-pig complement. No cytotoxic titre is observed with 1:10 diluted guinea-pig complement indicating that anticomplementarity cannot be reduced by enzyme treatment of the lymphocytes (Table I). However, by 3 successive washings of the sensitized lymphocytes anticomplementarity of mouse serum against guinea-pig complement can be removed so that the same titre is reached even with 1:10 diluted guinea-pig complement as with rabbit complement. If neuraminidase treated lymphocytes are incubated with antiserum and washed in the same way, a similar loss of anticomplementarity is noted. Interestingly, there is also a cytotoxic titre fall from 1:2048 to 1:256 if rabbit complement is used (Table II).

The extreme titre gap in these cytotoxicity tests with guinea-pig and rabbit complement can only partly be explained by anticomplementarity of mouse serum against guinea-pig complement. On the other hand, there could be an 'enhancing factor' in rabbit serum which interacts with neuraminidase treated sensitized lymphocytes. The results of RAY et al.[6], that rabbit complement treated with cobra venom was fully lytic for neuraminidase treated cells, may lead to an explanation for these striking results. Our postulated 'enhancing factor' cannot be identical with the known natural antibody against mouse lymphocytes, present in most rabbit sera[7], because our rabbit serum was twice absorbed on C 57 lymphocytes, rendering it nontoxic against neuraminidase treated C 57 lyphocytes. Furthermore, it is interesting and of practical consequence that the highest titre with neuraminidase treated lymphocytes and rabbit complement is obtained in the one step procedure, whereas usually the two step method is more sensitive than the one step procedure. It seems likely that the neuraminidase treated cell, which is thought to bind more antibody and complement because of a change in steric surface configuration[3,8], cannot bind this antibody so strongly as the untreated lymphocyte.

Table II. Reciprocal cytotoxic titres of anti C57 DBA serum with neuraminidase treated C57 lymphocytes in a two step cytotoxicity assay after 1 and 3 washings of the sensitized lymphocytes

Neuraminidase pretreatment of lymphocytes		
1 × washed	3 × washed	Absorbed complement
1056	256	rabbit
16	32	guinea pig, undiluted
4	16	guinea pig, 1:10 diluted

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Zusammenfassung. Es wird gezeigt, dass im Cytotoxizitätstest mit Mäuselymphocyten der cytotoxische Titer durch Neuraminidasevorbehandlung stark gesteigert werden kann, falls Kaninchenserum als Komplement verwendet wird. Durch Trypsin- oder Ficin-Vorbehandlung wird nur eine schwache Steigerung erzielt. Im Gegensatz zum Cytotoxizitätstest mit unbehandelten Lymphozyten wird der stärkste Titeranstieg bei der «one step»-Methode beobachtet. Um die spezifische Titererhöhung mit Kaninchen-Komplement zu erklären, wird ein «enhancing factor» mit einer Spezifität gegen Neuraminidase behandelte antikörperbeladene Lymphocyten postuliert.

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Anti-Lymphocyte Serum and Suppression of Interferon Production

Although the effect of heterologous anti-lymphocyte serum on interferon production has been studied by several investigators, there is no general agreement regarding the nature of this effect. HIRSCH et al.[1] reported no significant depression in interferon levels of mice given 3 injections of rabbit antimouse lymphocyte serum before stimulation with vaccinia virus. BORDEN et al.[2] obtained a slight reduction (2-fold) in interferon titers of mice treated with anti-lymphocite serum. In contrast to these findings, a study by BARTH et al.[3] reported a significant reduction in interferon titers of mice given 3 injections of burro antimouse lymphocyte serum. The present study reports additional data on suppression of interferon production by antilymphocyte serum.

Methods. Anti-mouse lymphocyte serum (ALS) was produced by immunizing rabbits with two i.p. injections of mouse thymocytes, according to a modified procedure of LEVEY and MEDAWAR[4]. Rabbits were bled 1 week after the last injection. Sera were pooled, inactivated at 56 °C for 20 min, and adsorbed with 20% mouse red blood cells for 1 h at 37 °C and overnight at 4 °C. Agglutination tests with mouse red blood cells were negative after adsorption. Pooled serum was tested for antibody to mouse lymphocytes by the cytotoxicity test: incubation of ALS with mouse spleen cells for 30 min at 37 °C resulted in 32% cell mortality, compared to 6% mortality in normal rabbit serum (NRS) controls. Mice were injected i.p. with 1 ml of either ALS or NRS, followed 24 h later by 1 ml Newcastle disease virus (NDV) (640 hemagglutinating units). Animals were bled 8, 16 and 25 h after injection of NDV. Sera were collected and assayed for interferon in L-929 cells by inhibition of cytopathogenic effect, using vesicular stomatitis virus as challenge[5].

Results. Treatment of mice with ALS produced a 4-fold reduction in the amount of interferon produced, when compared to NRS-treated controls. Suppression could be demonstrated 8 h after stimulation with NDV. After 24 h, interferon could not be detected in ALS – treated mice (Table).

These results are in general agreement with those of BARTH et al.[3] who reported a 4- to 5-fold reduction in the amount of interferon produced in ALS-treated mice.

Depression of interferon response in ALS-treated mice constitutes indirect evidence for involvement of lymphoid cells in interferon production.

Résumé. L'administration de 1 ml ALS, 24 h avant l'introduction de l'interféron avec le virus de la maladie de Newcastle, provoqua une réduction au quart du taux de l'interféron.

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Effect of treatment with ALS or NRS on induction of interferon in mice by NDV

Treatment	Interferon titer (units/3 ml) h after induction		
	8	16	24
NRS	1500	550	280
ALS	390	200	0

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Hemoglobin in Immature Erythrocyte Mitochondrion-Like Organelles

An increase in number of mitochondria occurs in reticulocytes after nuclear extrusion by erythroblasts in the peripheral blood of rabbit-embryos[1]. Mitochondrion-like organelles (MLO) in cells fixed in hypotonic medium and stained by phosphotungstic acid were shown from pinocytotic vesicle formation onwards[2]. Reticulocyte MLO are formed concomitantly with the transformation of iron containing material, for heme biosynthesis. Besides their higher number in relation to the later erythroblasts, reticulocyte MLO, constituted predominantly by longitudinal cristae, are highly electron dense, suggesting a high protein content in these organelles[2].