Varying responses of the erythrocytes of sheep, man, and rat to agents which induce 'hot–cold' hemolysis

Agent	Sheep	Man	Rat
a) I_3^-	2.9	7.3	7.0–22.0
b) *Cl. welchii* Phospholipase C	0.50	0.92	31.0
c) *S. aureus* (strain R-1) β-toxin (G. M. WISEMAN[9])	2048	256	<4

a) For I_3^- the data are expressed as the molar ratio I_3^-/RBC phospholipid causing the maximum hemolytic response of 0.3% RBC at pH 6.9 in 0.1 *M* potassium phosphate buffer during 60 min of incubation with the standardized amounts of I_3^- at 37 °C. b) For *Cl. welchii* phospholipase C the data are expressed as micro-g of enzyme which causes 50% hemolysis in pH 6.5 isotonic saline containing 2 m*M* $CaCl_2$, during 30 min incubation of 0.3% RBC at 37 °C. c) The data[9] are expressed as dilutions of a β-toxin preparation giving the same hemolytic response at similar conditions (hemolytic units/ml).

Cl. welchii phospholipase C which releases the phophoryl-nitrogenous moiety from phosphatides of the erythrocyte membrane[8] shows a differential response of the erythrocytes of sheep, man, and rat similar to that induced by I_3^- (Table), and – under proper conditions – the 'hot-cold' phenomenon.

Our results support a view that the fixed positive charge of phospholipids (in particular, sphingomyelin) may regulate the leakage of hemoglobin through the erythrocyte membrane. Once facilitated this passive movement of hemoglobin proceeds and reaches an equilibrium in the 'hot' phase. When the cells are transferred into the ice bath before the equilibrium is attained the passive movement of hemoglobin proceeds until the equilibrium is reached ('cold' phase of the classical microbiology). The effect of 'hot-cold' hemolysis appears to be a common feature of the response of erythrocytes to any agent which alters or removes $-N^+(CH_3)_3$ groups of membrane phospholipids.

Zusammenfassung. Nachweis der Wirkung bestimmter Exotoxine von Bakterien auf die Erythrozytenmembran, wobei die Änderung der Permeabilität durch die Abspaltung von Cholin aus den Phospholipiden der Membran erfolgt.

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Antigen-Induced Mitosis in Liver Macrophages of Immunized Mice

Activation of macrophages appears to be essential to the expression of acquired resistance to many experimental infections[1,2]. Activated macrophages differ from 'normal' (non-activated) macrophages in having a greatly increased capacity to destroy intracellular micro-organisms, increased rates of phagocytosis and spreading on glass and an increased content of phase-dense and phase-lucent granules[1,3]. The process of activation is cell-mediated and immunologically specific[1,2,4]. Activation and the acquisition of resistance are generally accompanied by the development of delayed-type hypersensitivity[5] and the occurrence of mitosis in the macrophages of the peritoneal cavity[6], spleen and liver[7,8]. It has also been reported that mitosis can be induced in peritoneal macrophages by the re-injection of a soluble pure protein antigen into mice previously immunized with that antigen in Freund's adjuvant[9]. We have confirmed this observation; shown that the induction of mitosis is immunologically specific and is accompanied by morphological signs of activation; observed that mice which respond in this way also have delayed-type hypersensitivity to the antigen; and found that mitotic reactivity could not be conferred on normal mice by injection of high-titred antisera from immunized mice or rabbits[10]. In the course of these experiments we wished to determine whether the hepatic macrophages (Kupffer cells) which turn over very slowly – about once every 60 days[11] – and are thus a relatively fixed population, would also respond mitotically to antigenic challenge in immunized mice.

Non-inbred female SW mice were immunized by s.c. injections, 1 week apart of 100 µg of 4×recrystallized human serum albumin (HSA, Nutritional Biochemicals Corp.) as an emulsion of equal volumes of a saline solution with Freund's complete adjuvant (Difco). 4 weeks after the 2nd injection they received a s.c. injection of 2 mg HSA in 0.2 ml saline. Controls comprised immunized animals injected with saline only and non-immunized animals injected with HSA or saline. 20 h later they were injected i.v. with 100 µg vinblastine sulphate (Velbe, Lilly) in 0.5 ml saline. After a further 3 h they were killed by cervical dislocation and portions of the livers were removed and fixed in formol-saline. Sections 6 µm thick were cut from paraffin embedded blocks and stained with haematoxylin and eosin. The number of macrophages in mitosis was counted in 100 microscopic fields at a magnification of 100×. The abnormal mitotic figures were easily recognizable and those in Kupffer cells were distinguishable from the rare mitoses in hepatic parenchymal cells. In order to ensure that the results were not affected by distortion or compression of the tissue during processing,

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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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