Further Evidence Concerning Macrophages Producing 19 S-Antibody in Mice

In a previous communication we described macrophages which produced in mice 19 S-Antibody against SRBC[1]. This result was obtained by a modification of the plaque technique of JERNE and NORDIN[2] and a morphological study of the plaque-forming cells[1]. In further experiments we combined cytological observations with functional studies on these plaque-forming cells.

The morphological aspect of the plaque-forming cells indicates that some of these cells are macrophages. If it is so, then these cells should demonstrate phagocytic activity. The purpose of this study is therefore to show that plaque-forming cells engulf erythrocytes and that they can phagocytize particles while producing antibodies.

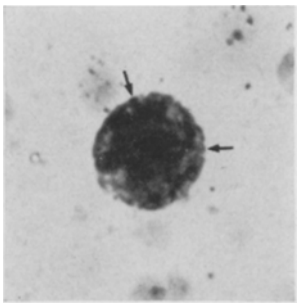

Fig. 1. Plaque-forming cell with phagocytized ink particles in the cytoplasm (small dark points, 2 indicated by arrows. Pappenheim-staining. × 1850.

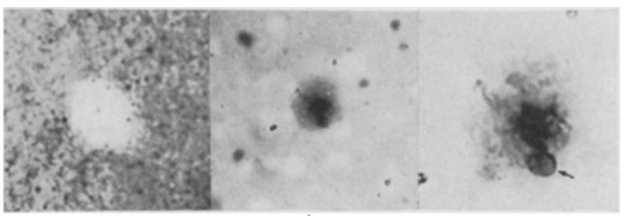

Fig. 2. Plaque-forming cell, 3 days after immunization: phagocytized erythrocyte and ink particles in the cytoplasm (arrow). Pappenheim-staining. a, × 180; b, × 950; c, × 1850.

NMRI mice, random bred, females weight 25 g were immunized i.v. against 10^8 SRBC. The mice were killed 3 and 4 days after immunization and the plaque-forming cells prepared[1,2]. 12 h before sacrifice the mice were i.v. injected with 0.2 ml of a 25% solution of Indian ink in saline (Günther Wagner, Hannover, West-Germany, 21 A 896, particle size 20–60 nm). In another experiment the prepared spleen cells of immunized mice were incubated in vitro with 1 ml of a 1% solution of Indian ink for 30 min at room temperature. After the incubation time the plaque assay was performed. The number of plaque-forming cells was not influenced by the exposure to the Indian ink. The cells were stained by the Pappenheim-method.

Results. (1) Plaque-forming cells had phagocytized ink particles in vitro. The uptake was observed mostly in cells with a large nucleus with most basophilic cytoplasm (Figure 1). (2) Plaque-forming cells exposed to ink particles in vivo incorporated these particles in their cytoplasm. (3) Furthermore we observed that plaque-forming cells phagocytize not only ink particles but also erythrocytes (Figure 2).

These observations show that antibody producing cells in the early immune phase can perform phagocytosis during the antibody production[3].

Zusammenfassung. 19-S-Antikörper-bildende Zellen können während der Antikörperbildung in der Frühphase einer Immunantwort wie Makrophagen phagozytieren.

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[1] H. NOLTENIUS and P. RUHL, Experientia *25*, 75 (1969).
[2] N. K. JERNE and A. A. NORDIN, Science *140*, 405 (1963).
[3] The work is supported by grants from the Deutsche Forschungsgemeinschaft and the Stiftung Volkswagenwerk.

IgG as the Main Immobilization Factor in Rabbit Antiserum Against *Entamoeba*

That heat-inactivated hyperimmune rabbit sera were capable of immobilizing *Entamoeba histolytica*, was initially demonstrated by COLE et al.[1] Tests with unheated sera were oberved to cause lysis of the amoebae[2], an observation probably indicating that complement plays a role in the lytic activity of various microorganisms[3,4]. Unlike immobilization of bacteria[5], the immobilization of the *Entamoeba* sp. is reversible[6]. BIAGI et al.[7], using the immobilization reaction (IMR), demonstrated that the immobilization factor produced by the host after contact with the amoeba was the γ-globulin fraction. This paper presents evidence which show that, in hyperimmune rabbit sera, IgG is largely responsible for the immobilizing activity in 3 species of *Entamoeba* tested.

Material and methods. E. histolytica, E. moshkovskii and *E. invadens* were cultivated in a monophasic medium according to JONES[8], the first being incubated at 37 °C and subcultured every third day, while the latter 2 species were incubated at room temperature (26 °C) and subcultured every seventh day. The amoebae were harvested by centrifugation at 2500 rpm for 3 min and then washed 3 times with normal saline to rid them of most bacteria before inoculation into healthy adult rabbits. About 3,000,000 amoebae were injected on each occasion. The injections – twice s.c., followed by 3 times i.v., with a final booster dose of amoebae in Freund's complete adjuvant – were spaced at 5-day intervals. The animals were bled by the marginal ear veins 10 days after the