A Simple Method for Evaluation of Latex Phagocytosis by Rat Peritoneal Macrophages

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Phagocytosis is an important function of polymorpho-nuclear cells and macrophages, closely related to the immune defense of the organism. Phagocytic cells engulf not only bacteria, but also other cells, as well as inert particles, such as latex. One method for evaluation of phagocytosis is based on incubation of phagocytic cells with latex particles in vitro at maximal incubation conditions which require not only careful isolation and purification of the cells, but also applying meticulous techniques for maintaining constant temperature, suitable pH, and incubation media [1]. Even if the incubation is carried out under the best possible conditions, the withdrawal of the cells from their physiological milieu presents a serious trauma which may impair their phagocytic capacity. Hereby, we suggest a simple method which permits in vivo evaluation of the phagocytic function of rat peritoneal macrophages and polymorphonuclear cells under maximal, almost physiological conditions. An amount of 5 ml of a 5 % suspension of uniform polystyrene latex particles (0.8 μ m in diameter; Difco, Detroit, Mich., USA) was injected into the peritoneal cavity of Wistar rats weighing 330-400 g. The animals were kept in their cages at room temperature for 60 min. To obtain the cells, the peritoneal cavity was 'flushed' by injection of 60-80 ml of phosphate-buffered saline, followed by gentle shaking and rotation of the animals for 5 min. The intraperitoneal fluid was withdrawn, and the cells were sedimented by centrifugation at 250 g for 10 min. Smears stained by the May-Grünwald-Giemsa method were examined under a

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Fig. 1. Smears from peritoneal cells containing latex particles.

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Fig. 2. Semifine sections from embedded peritoneal cells with engulfed latex particles.

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light microscope, and the number of latex particles engulfed by the cells was detected. A few cells containing latex particles are shown in figure 1.

To avoid superposition of the latex particles on the cells, they may be fixed in cold 1 % glutaraldehyde in cacodylate buffer (pH 7.4) and embedded in agar 100 resin (Agar Scientific, Cambridge, UK) or another polymer using standard methods for electron microscopy.

Examination of semifine sections stained with methylene blue under the light microscope permits more precise evaluation of the number of latex particles phagocytosed by the cells (fig. 2). Thin sections could be used for electron microscopic examination. The cells shown in the figures seem to be overloaded with latex, since the experiments were carried out for demonstration purposes. Injection of

a lesser amount of latex would permit easier counting of the engulfed particles.

The method described is simple, avoids cell trauma as much as possible, and permits in vivo 'incubation' of the cells with the latex particles under almost physiological conditions. The animals did not show any signs of distress.

Reference 1 Stevenson GW, Hall SC, Miller PJ, Alvord G, Leventhal JB, Seleny F, Stevenson HC: The effect of anesthetic agents on human immune system function. I. Design of a system to deliver inhalational anesthetic agents to leukocyte cultures in vitro. *J Immunol Methods* 1986;88: 227-283.

Announcement

Second European Day against Leukemia and Lymphoma

June 21, 1997

Following the very encouraging outcome of last year's edition, the second European Day against Leukemia and Lymphoma is underway. A chain of events will be organized in all European countries by the numerous associations which operate in support of research for hematological malignancies and in assisting our patients.

The Italian Association against Leukemia (AIL) has contacted the major associations operating in this field and established a program aimed at publicizing this initiative. The promotion of the European Day will include the following:

Printing and distribution of informative material to hematological centers, nonprofit organizations, congresses and meetings.

Production of a specific advertisement for newspapers and magazines.

Participation at television shows. The European Day will be publicized in important television talk shows. RAI will broadcast the second edition of Angels under the Stars, a program specially dedicated to this event.

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A Simple Method for Evaluation of Acta Haematol 1997;98:56-57 57

Phagocytosis