# Demonstration of cross reaction between anti-macrophage antibodies and mononuclear mesodermal cells[1]

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*Summary.* An anti-macrophage antiserum to rat peritoneal macrophages was prepared in rabbits. The antibodies produced showed cross reaction with perivascular adventitial macrophages, with macrophages in thymus and spleen, and with brain microglial cells.

The central problem in analyses of the posttraumatic healing mechanisms is the origin of brain macrophages. Some of the phagocytic functions are performed by neutrophilic leucocytes[2], but phagocytosis, especially in the later part of healing of brain injuries, is also performed by hematogeneous monocytes[3-5]. However, there is still doubt about the origin of many reactive cells in the brain[5-9].

Many attempts have been made to characterize the reactive cells in different kinds of cerebral injury. Isotope-labelling studies have shown that at least part of the reactive phagocytes in brain injuries associated with gross blood brain barrier damage consists of hematogeneous monocytes[10-12]. In lesions without blood brain barrier injury, e.g. hypoglossal nerve transection, isotope-labelling studies indicate that at least part of the reactive perineuronal 'microglial' cells are also of hematogeneous origin[13].

As isotope as well as enzyme histochemical studies, and silver impregnation studies[14], only give indirect evidence of the origin of reactive cells in the brain, the present study was performed to characterize and elucidate further the origin of reactive macrophages invading injured areas in the brain.

*Materials and methods.* Rat peritoneal mononuclear macrophages were prepared by rinsing the rat peritoneal cavity with tissue-culture medium 199 for 2 min; the cells recovered were centrifuged and washed repeatedly with medium 199. The cells were counted in a chamber, and $10^7$ cells were suspended in 1 ml saline, frozen and homogenized. 1 ml homogenate (of $10^7$ cells) was mixed with an equal volume of Freund's complete adjuvant, and injected into the hind subcutis of adult rabbits: $10^7$ cells once weekly for 4 weeks, and after a further 4 weeks an i.v. injection of $10^7$ cells without Freund's adjuvant. 10 days after the i.v. injection, the rabbits were given s.c. injections once weekly for 5 weeks. 1 month after the last s.c. injection, the rabbits were given an i.v. booster injection of $10^7$ homogenized cells without adjuvant. 10 days after the last injection, the rabbits were bled and serum prepared. The IgG fraction of serum was precipitated by ammonium sulphate and frozen after repeated washing.

Rats of the Sprague-Dawley strain with weights of 200–220 g were anesthetized with ether, and a frontal cerebral stab wound was made with a steel needle[14]. After 1–8 days, the rats were anesthetized with ether and transcardially perfused with 4% formaldehyde in 0.1 M phosphate buffer, followed by immersion in the same fixative for 12 h. Parts of the CNS, liver, spleen, kidney, lung and thymus were sectioned and preparation of peritoneal macrophages was performed by peritoneal washings. Blood monocytes were prepared by Ficoll-Hypaque gradient centrifugation.

Frozen sections of the tissues were incubated in anti-macrophage anti-serum (dilutions 1:5–1:30 in 0.1 M phosphate buffer with 2% bovine albumin) for 45 min, followed by repeated washings in buffer, and then incubated with goat-IgG against rabbit IgG (Miles Lab. Elkhart, USA) labelled with tetramethylrhodamin isothiocyanat (TRITC), diluted 1:5–1:30 in 0.1 M phosphate buffer with 2% bovine albumin, pH 7.2, for 30 min. After rinsing, the sections were mounted in 50% glycerol in phosphate buffer and studied and photographed in a Leitz light and fluorescence microscope with standard exitation and barrier filters for TRITC.

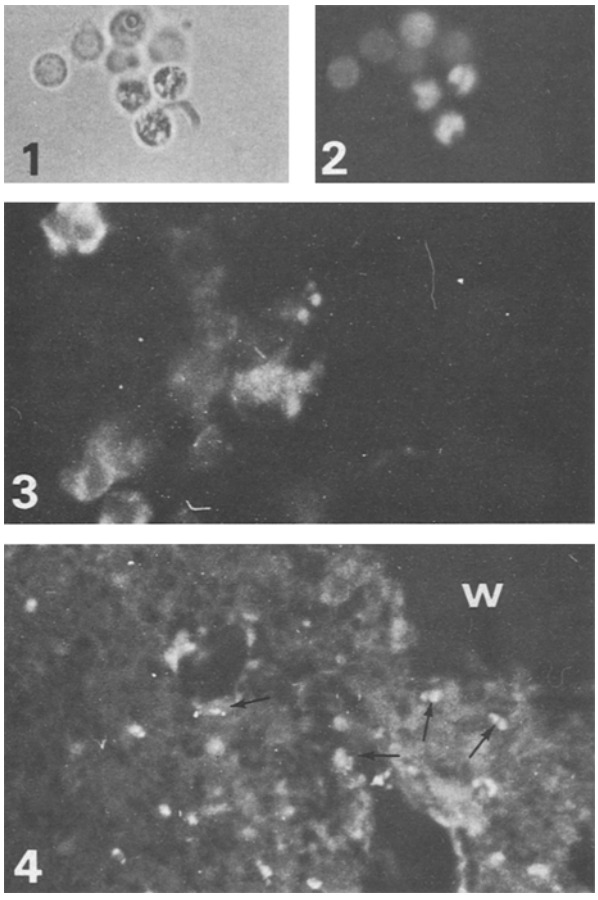
Rabbit antisera to albumin, as well as preimmune sera from the rabbits, were used as controls of the specificity in the 1st incubation. TRITC-labelled antisera to albumin, S-100 protein and 14.3.2 protein were used as specificity controls in the 2nd step. Anti-macrophage IgG, repeatedly absorbed with small amounts of homogenized peritoneal macrophages, were incubated at 37 °C for 60 min, stored at +4 °C overnight, and centrifuged at 10,000 × g for 30 min, was used as a negative control.

*Results and discussion.* The specificity of the rabbit anti-macrophage IgG was tested against different organ extracts, and the respective antigens of peritoneal macrophages by immunodiffusion in agar by the Ouchterlony technique. Anti-macrophage activity, as tested by the Ouchterlony technique, was observed in spleeen, liver, kidney, thymus and lymphe nodes, but only in hardly detectable amounts in normal nervous tissue. There was one major precipitation line in the Ouchterlony gel in all organs tested; but in peritoneal macrophages and kidney, there was also a second very weak precipitation line.

Specific immunohistochemical localisation of the antigens was observed by fluorescence microscopy using the TRITC-labelled goat-anti-rabbit IgG antiserum. There was an intense cytoplasmic fluorescence in peritoneal macrophages (figures 1 and 2), and in hematogeneous monocytic cells. There was a good correlation between the distribution of non-specific esterases and the distribution of the specific fluorescence (figures 1 and 2). Hematogeneous neutrophilic leukocytes, as well as thrombocytes and lymphocytes, showed no specific anti-macrophage activity. In the thymus there was an intense specific fluorescence in the small clusters of mononuclear cells at the margin of the thymic germinal centres, while the stroma macrophages (reticular cells) and fibroblasts showed no specific staining. In the spleen, clusters of ovoid mononuclear cells with specific anti-macrophage fluorescence, were observed both in the red and in the white pulp, especially along larger vessels. Mononuclear macrophages, showing specific fluorescence in their cytoplasm, were also observed in the adventitial sheath of larger vessels in spleen, liver, kidney, thymus (figure 3) as well as in the leptomeninges of the brain. Siderophages of the spleen and the Kupffer cells of the liver showed no specific fluorescence.

In cerebral stab wounds, anti-macrophage activity was observed in reactive monocytic macrophages, similar to microglial cells, invading the peritraumatic brain parenchyma (figures 4 and 5). These cells were occasionally observed in large clusters around venules and capillaries during the first postoperative week. Neutrophilic leucocytes invading brain parenchyma showed no specific fluorescence.

Our study shows cross reaction between an antiserum to rat peritoneal macrophages and mononuclear macrophages in thymus, spleen and around blood vessels, of a variable differentiation grade, but also in blood monocytes as well as peritoneal macrophages and microglial cells in cerebral stab wounds. The findings indicate a common mesodermal origin of phagocytic mononuclear cells, although their



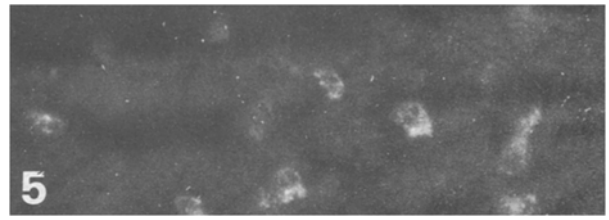




Fig. 1. Rat peritoneal macrophages 5 days after i.p. injection of paraffin. After formaldehyde fixation the cells were incubated with rabbit antiserum against rat peritoneal macrophages, followed by incubation in TRITC-labeled goat antiserum against rabbit IgG. Then, the cells were incubated with naphthyl-AS-BI-phosphate and Fast Red TR salt to demonstrate nonspecific esterases. There is an intense activity of nonspecific esterases in the cytoplasm of most peritoneal macrophages. Light microscopy, unstained. ×580. Fig. 2. Rat peritoneal macrophages as shown in figure 1. Fluorescence microscopy to demonstrate TRITC-fluorescence. There is a moderate cytoplasmic fluorescence, indicating the presence of antigens reactive with the anti-macrophage antiserum. There is a close correlation between the localisation of nonspecific esterases, as shown in figure 1, and the anti-macrophage fluorescence in figure 2, indicating a true cytoplasmic distribution of the antigen. ×580. Fig. 3. Rat thymus incubated to show macrophage-specific antigens. There is an intense cytoplasmic fluorescence of TRITC in several macrophages. The surrounding thymocytes display no specific fluorescence. Fluorescence microscopy. ×950. Fig. 4. Cerebral stab wound 3 days after injury, incubated with anti-macrophage antiserum. Several small cells in the immediate surrounding of the stab wound (W) display an intense cytoplasmic fluorescence of TRITC (arrow). Fluorescence microscopy. ×230. Fig. 5. Fluorescent macrophages surrounding a cerebral stab wound in a rat 3 days after injury. The fluorescence is cytoplasmic and there is no fluorescence in neurons or neuroglial cells. Fluorescence microscopy. ×580.

differentiation into blood monocytes, histiocytes, etc, results in a variable ultrastructural appearance. Studies are in progress to characterize further the antigens and the antiserum, including localisation at the cellular and subcellular level.

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## HBsAg uptake by macrophages in vitro: An immunofluorescence study[1]

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*Summary.* The uptake of HBsAg by in vitro cultured macrophages was studied by immunofluorescence method. Intracytoplasmic fluorescent particles appeared 3 h after the contact with HBsAg-positive serum, while after 24–48 h only a few cells contained these particles, which are probably destroyed within the cytoplasm.

Australia antigen (HBsAg) is a particle strictly associated with serum hepatitis[3], which does not contain nucleic acids, so that it has been considered as a defective virus or an antigenic determinant of a virus able to cause hepatitis[5,6]. It is well-known that the presence of HBsAg in the serum is directly related to its localization in the liver cells, while other tissues, such as bone marrow, kidney and cells obtained from bile and duodenal drainage, do not contain