Extracellular Localization of a Protein Antigen in Lymphatic Tissues in Contact with Lymphocytes

Corpuscular antigen in lymph nodes has been found on electronic optical level not only intracellularly in macrophages but also associated with surface of dentritic processes of reticular cells in contact with lymphocytes[1]. This result and previous investigations by NOSSAL et al.[2,3] led to speculations that are in contrast to the opinion of FISHMAN[4] and other authors[5,6] about the role of macrophages in the process of antibody production.

The investigations of MITCHELL and ABBOT[1] have been performed autoradiographically with J^{125} labelled flagella. This method does not give direct evidence of the antigen itself but only of J^{125}. This may lead to an erroneous interpretation of the results for 2 reasons: firstly, it is not guaranteed that J^{125} labels the determinant groups of the antigen; secondly, it cannot be excluded that during the experiment J^{125} will be split off from the antigen. The radioactivity found in the thyreoid tissue during the experiment supports the second objection in regard of splitting off, at least, some label from antigen[3].

Horse-radish peroxidase (HPOD) is a water-soluble protein antigen. It is immunogen for rabbits[7,8]. The localization of this enzymeprotein in lymphoid tissues such as spleen, lymph nodes and tonsils has been investigated by light microscopy[9,10]. HPOD is also adopted for electron microscopic detection in various tissues[11]. Compared to an autoradiographic method, this method has the advantage of detecting the intact antigen molecule, because the detection of HPOD is carried out by its enzyme activity. 3 kg New Zealand rabbits received i.v. 100 mg HPOD. Previous investigations about the clearance of HPOD indicated that 3 h after the injection HPOD is removed from the bloodstream. Therefore, the animals were killed 3 h after the injection.

Spleen and lymph nodes were fixed for 5 h at room temperature in a formaldehyde-glutaraldehyde fixative[11]. The tissue blocks were then washed briefly in cold, distilled water. They were then frozen and cut on a Linde freezing microtome. Sections 10 μ and 40 μ in thickness were incubated in KARNOVSKY medium[11] containing 0.75 m*M* 3.3-diaminobenzidine tetrahydrochloride in 0.05*M* *Tris* buffer pH 7.4 and 0.01% H_2O_2 for 15 min. After incubation the sections were washed in distilled water and then postfixed for 1 h in 1% osmium tetroxide buffered to pH 7.4 with 0.1 *M* [cacodylate] buffer. 10 μ sections were dehydrated in xylole and embedded in Eukitt for light microscopic examination. For electron microscopy 40 μ sections were dehydrated in acetone. Ultrathin sections were cut with a LKB-ultrotome and examined under a Siemens Elmiskop I with and without poststaining with uranyl acetate and lead citrate. Controls: (1) fixed-frozen sections of tissue from rabbits injected with peroxidase were incubated in media which omitted either the hydrogen peroxide, or the diaminobenzidine, from the otherwise complete incubation media; (2) fixed-frozen sections from tissue of uninjected rabbits were incubated in the complete medium.

Under these conditions granylocyts and erythrocyts also give a positive reaction because of their endogen peroxydase activity. HPOD is detectable by light microscopy only as phagocyted material in granules of reticular cells. The reticular cells of the venous sinuses and of the red pulp of the spleen have phagocyted HPOD to a high degree. In the reticular cells of the lymphatic nodules of the spleen, HPOD is absent or only in very few cases detectable. The reticular cells of the sinuses and of the medullary cords in the lymph nodes phagocyted HPOD considerably; the reticular cells of the lymphatic nodules, however, only in very few cases. These results conform with the findings described by SAUER[10].

Beyond these findings extracellular HPOD, too, can be detected with electron microscopy. It is, however, limited to certain regions. In contrast to controls the extracellular space is filled with extremely electron-dense material (Figure 1). This material is often so close to the cell membranes that the membranes themselves no longer appear separately. Extracellular HPOD is visible to the highest degree in the venous sinuses and in the red pulp spleen. In the centre of lymphatic nodules of the spleen, no extracellular HPOD was observed. Lymphocytes and reticular cells are surrounded by HPOD in the red pulp and in the periphery of lymphatic nodules. A preferred localization to a certain kind of cells or to certain regions on the surface of the cells is not detectable. Reticular cells also phagocyted extracellular HPOD. Much of the phagocyted HPOD is associated with large granules and vacuoles in these cells.

In lymph nodes HPOD is detectable extracellularly only in the medullary cords. Also in this case lymphocytes, as well as reticular cells, are directly associated with HPOD (Figure 2).

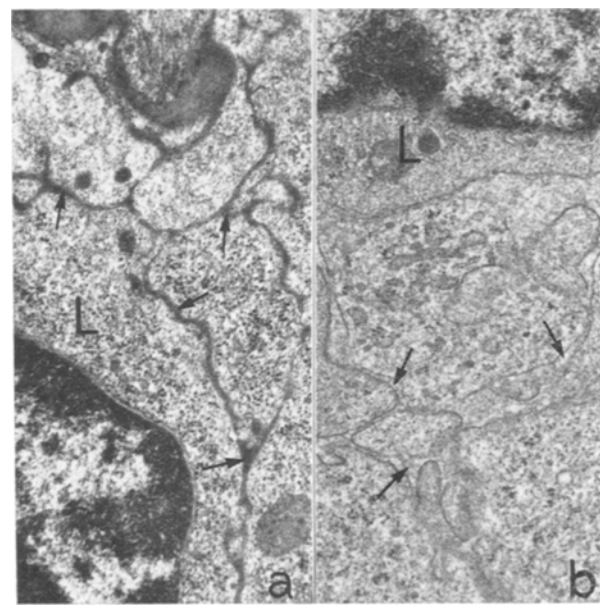

Fig. 1 (a and b). Spleen of rabbit. L, lymphocyte; ↑, intercellular space. (a) Positive reaction. (b) Control, × 28,000.

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In contrast to flagella[1] HPOD fills the intercellular space between lymphocytes and also between lymphocytes and reticular cells. Antigen material is associated directly with the membranes of these cells. From this point of view, consequently, there exists the possibility that unchanged antigen material may react directly with receptors on lymphoid cells without earlier 'processing' in macrophages. Previous in vitro investigations[12] have shown that even a few seconds of antigen contact with lymphoid cells are able to induce a specific transformation and proliferation of these cells.

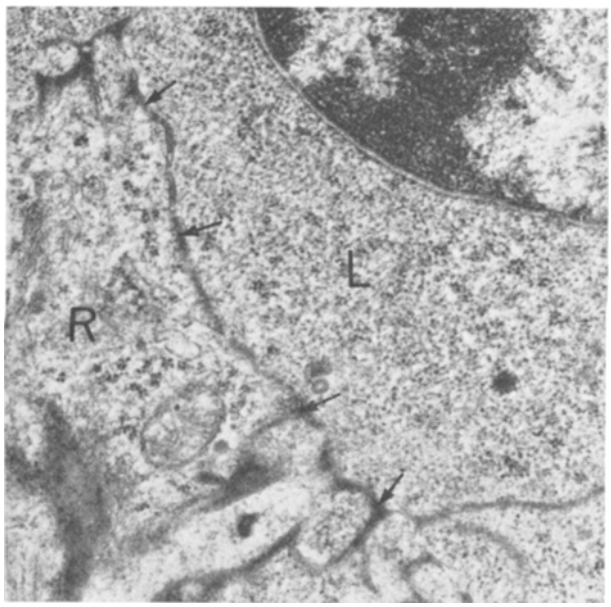

Fig. 2. Lymph node of rabbit. L, lymphocyte; R, reticular cell; ↑, positive reaction in intercellular space, × 35,000.

Zusammenfassung. Drei Stunden nach i.v.-Gabe an Kaninchen ist das Antigen Meerrettich Peroxydase extrazellulär in den Marksträngen der Lymphknoten und der roten Pulpa der Milz elektronenmikroskopisch nachweisbar. Das unveränderte Antigen steht damit noch zu dieser Zeit in direktem Kontakt mit der Zellmembran der lymphoiden Zellen.

T. DIAMANTSTEIN, B. WAGNER and D. GRAF KEYSERLINGK

Klinikum Steglitz of the Free University Berlin, Department of Oto-rhino.-laryngology, Biochemical and Immunological Laboratory, D-1 Berlin 33, and II. Anatomisches Institut of the Free University Berlin, (Germany), 9 July 1969.

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The Cellular Sources of Antibodies in Diffusion Chamber Cultures of Alveolar Exudates

Diffusion chambers (DC) are a valuable tool for the study of immune processes in closed cellular systems[1,2]. The chambers are implanted in new-born or irradiated recipients which are incapable of demonstrable antibody production to the amount of antigen applied. In competent adult recipients DC have a strong adjuvant effect on the recipient's response against soluble antigens[3]. Although new-born recipients do not respond serologically to the antigens enclosed in the DC[1], the cellular events occurring in their lymphatic tissues under the influence of the DC cultures of antibody forming cells deserve closer attention. In the present study lung alveolar exudate cells were cultivated in the DC in newborn rabbits, since 'adult' histiocytes may enhance the recipient's immune capacity[4].

Methods. Alveolar cells were washed out from lungs of young adult chinchilla rabbits in Earle's fluid. The donors were either untreated, or had been immunized intratracheally with $2–4 \times 10^9$ sheep red blood cells (SRBC) 8–30 days previously. The Earle-suspended alveolar cells were mixed with SRBC (ratio 1:2) and 1 ml of the suspension injected into DC made from lucite rings and millipore filters of 0.1 μ porosity. The filled DC were implanted into the peritoneal cavities of 5 days old chinchilla rabbits. Control animals received DC which contained either SRBC or alveolar exudate cells from untreated donors. In the latter case the appropriate amount of SRBC was injected i.p. into the recipients of DC.

After 8 days (cultures of preimmunized cells) or after 9–10 days the DC were removed from anaesthetized recipients. The cultured cells were liberated from the DC by mechanical scraping or by pronase treatment[2]. Neither technique detached all cells from the filters. Recipient's lymph was collected from cisterna chyli (yield $1–1.5 \times 10^7$ viable lymph cells). Then the recipients were killed and the samples of the lymphatic tissues and omentum-derived tissue covering the DC taken out and teased in Earle's fluid (yield $1.2–10 \times 10^7$ viable cells per organ or tissue). All cell samples were washed, suspended in Sevac IV medium and assayed for plaque-forming cells (PFC) by JERNE's technique[5]. The PFC were examined microscopically in situ, after 2.5% glutaraldehyde fixation and methyl green-pyronin staining. The DC fluid and recipient's sera were tested by the standard hemagglutination and hemolysis technique in test-tubes.

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