SUBMICROSCOPIC DETECTION OF IMMUNOGLOBULIN RECEPTORS ON LYMPHOCYTE SURFACE MEMBRANES

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One of the main criteria for the detection of B lymphocytes is the presence of immunoglobulin receptors on their surface which can be found by various methods [1]. One promising method of identification of B lymphocytes is the discovery of immunoglobulin receptors on the surface of cells by means of antibodies against surface immunoglobulins or against their $F(ab')_2$-fragments [5, 11]. These antibodies, conjugated with horseradish peroxidase as label, can be used to identify B lymphocytes under the light-optical microscope [8, 9]. The sensitivity of the method using a horseradish peroxidase label has been shown to be higher than that of the luminescence method, and the end product of the reaction has fairly high electron density, so that this method can be used in electron-microscopic studies [3]. Nevertheless, up till now there have been few investigations of the ultrastructural identification of B lymphocytes and the methods so far used do not permit their characteristic morphological features to be established.

The object of this investigation was to study the subcellular localization of immunization of immunoglobulin receptors of surface membranes of human lymphocytes by the use of an immunoperoxidase method, which provides a sound basis for the drawing up of criteria for the ultrastructural identification of these cells.

EXPERIMENTAL METHOD

Heparinized venous blood from 10 healthy persons, bone marrow obtained by puncture from five blood donors, and tissue of the palatal tonsils of five patients with chronic tonsillitis, removed surgically, were used. Peripheral blood lymphocytes were isolated on a Ficoll-Verografin gradient [5]. The duration of the lymphocyte isolation procedure when this method was used often led to the development of destructive changes in the cell organelles. Accordingly in most cases a leukocyte concentrate, obtained by centrifugation for 10 min at 1000 rpm, was used. The lymphocyte suspension, fragments of bone marrow, and frozen sections of the palatal tonsils were fixed in 1% glutaraldehyde solution in phosphate buffer (pH 7.3) for 30 min. After careful washing in phosphate buffer the samples were incubated for 60 min with antiserum against human IgG, IgM, and IgA, conjugated with horseradish peroxidase. Conjugation of the monospecific antisera with horseradish peroxidase was carried out by Avrameas' method [2]. At the end of incubation films were prepared from the suspension of blood lymphocytes and used for light-optical microscopy. Peroxidase was detected in films, in the suspension of the blood lymphocytes, bone marrow fragments, and tonsil sections with the aid of 3,3-diaminobenzidine [4]. After the reaction for peroxidase the samples were postfixed with 1% OsO_4 solution in phosphate buffer for 60 min, dehydrated in alcohols, and embedded in Araldite. Some ultrathin sections were investigated without additional staining, others were stained with lead citrate. Sections were examined in IEM-100B and IEM-100S electron microscopes. Samples treated with horseradish peroxidase but without immune serum and with serum against rabbit globulins conjugated with horseradish peroxidase, served as the control.

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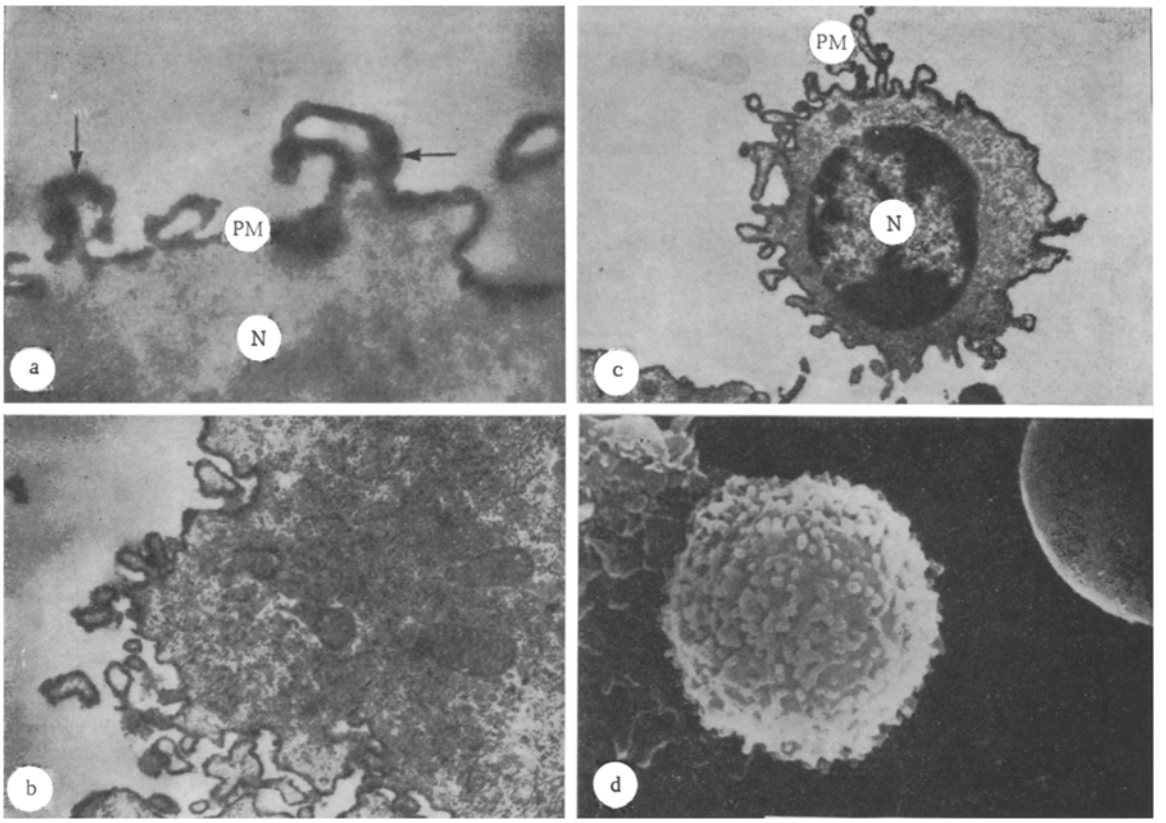

Fig. 1. Location of PRP and scanning electron microscopy of lymphocytes: a) deposition of reaction product on plasma membrane (PM) of blood lymphocytes, more intensive reaction is observed on outgrowths of PM (arrows). N) Nucleus. Ultrathin section, unstained, 35,000 ×; b) bone marrow lymphocyte with numerous outgrowths of PM, where dense reaction products can be seen, 8500 ×; c) deposition of reaction product on PM of bone marrow plasma cell, 14,000 ×; d) surface relief of presumptive blood B lymphocyte, distinguished by the presence of numerous microvilli. Scanning electron microscopy, 5000 ×.

EXPERIMENTAL RESULTS

Examination of films prepared from the blood lymphocyte suspension showed that the peroxidase reaction product (PRP), indicating the presence of immunoglobulin receptors, was distributed as a characteristic border on the cell surface. The number of lymphocytes giving a positive reaction on their surface varied from 15 to 25% of the total number, in agreement with data on the number of B lymphocytes detectable by the EAC rosette formation method [1]. The PRP, incidentally, also was found on the surface of monocytes, on account of nonspecific adsorption of immunoglobulins by receptors for Fc-fragments of antibodies [9]. In the electron microscope, blood lymphocytes carrying a homogeneous dense reaction product on their surface constitute a heterogeneous population as regards their ultrastructural organization. Most lymphocytes had numerous outgrowths of their plasma membrane similar in type to clasmacytosis, and their cytoplasm contained numerous free ribosomes and polysomes. The distribution of PRP varied over the cell surface. The most intensive reaction was found on those areas of cells where outgrowths of the plasma membrane were observed (Fig. 1a). Besides these cells, PRP also was found on the surface of lymphocytes with a relatively smooth plasma membrane, containing well-developed structures of a lamellar complex and single tubules of the rough endoplasmic reticulum in their cytoplasm. A positive reaction was found in the bone marrow on the surface of nearly all medullary lymphocytes. Just as in the blood, the most intensive deposition of PRP was observed on cytoplasmic outgrowths of lymphocytes and plasma cells (Fig. 1b, c). The surface relief of the lymphocytes, as examination with the scanning electron microscope showed, differed for T and B lymphocytes [10]. T Lymphocytes were characterized by a relatively smooth surface, whereas B lymphocytes had mainly a more complex relief with numerous microvilli (Fig. 1d). It can accordingly be postulated that blood and

bone marrow lymphocytes carrying immunoglobulin receptors, identified by the immunoperoxidase method, on their cytoplasmic outgrowths, consist of a population of B lymphocytes. It must be recalled that the presence of receptors for Fc-fragments on other cells besides B lymphocytes [6, 8] reduces the specificity of detection of the B cells. To prevent the antibody molecule from binding with the Fc-receptors of the cells the horseradish peroxidase must be conjugated with $F(ab')_2$-fragments of the antibodies, and this is a matter for special study. Despite this shortcoming, the method of detection of immunoglobulin receptors on the surface of lymphocytes by means of monospecific antisera conjugated with horseradish peroxidase is perfectly suitable for the identification of B lymphocytes at the light-optical and ultrastructural levels. The results obtained show that lymphocytes carrying membrane-associated immunoglobulin receptors (B lymphocytes) constitute a morphologically heterogeneous population, and this fact must be borne in mind when cells of this type are identified.

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ULTRASTRUCTURAL LOCALIZATION OF THE TYPE-SPECIFIC ANTIGEN OF *Legionella pneumophila*

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The discovery of a new genus of bacteria, *Legionella* (the etiologic agent of a hitherto unknown group of serious acute respiratory diseases in man) has generated a considerable volume of research aimed at studying the cultural, morphological, and biochemical properties of this agent [4, 6, 7, 9]. In particular, the study of the cellular antigens of the microorganism and their role in the mechanism of pathogenesis and immunity in experimental infection is most interesting [4, 8, 10]. Several type-specific and group-specific antigens have been isolated from avirulent strains of *L. pneumophila* and it has been shown that they can be used for experimental vaccination of laboratory animals; their biochemical nature has been studied [18].

A type-specific thermolabile antigen III was isolated previously from a virulent culture of *L. pneumophila* [1]. This antigen, unlike other antigens isolated from avirulent strains described previously, was toxic for a culture of guinea pig peritoneal macrophages *in vitro*,

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