Macrophage-lymphocyte clustering in rheumatoid arthritis

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Webb, F. W. S., Baker, M., Weisbart, R., Bluestone, R., and Goldberg, L. (1975). *Annals of the Rheumatic Diseases*, 34, 38. Macrophage-lymphocyte clustering in rheumatoid arthritis. The cells in synovial fluid from patients with rheumatoid arthritis contain a small percentage of macrophages. Such macrophages were isolated and cultured alone and with homologous and heterologous lymphocytes for 24 hours, in an attempt to identify possible contact between living lymphocytes and macrophages. Such contact was found, with clustering of lymphocytes around macrophages, and was particularly well shown by scanning electron microscopy.

Although some kind of autoallergic process has been put forward to explain the remarkable chronicity of the inflammatory process in such conditions as rheumatoid arthritis, recent work has, on the other hand, suggested that very small amounts of foreign antigen may persist in an active form in experimental models which resemble human rheumatoid disease. For example, Webb, Ford, and Glynn (1971) were able to show that minute amounts of antigen persisting in the joints of normal rabbits gave rise to a chronic synovitis if these rabbits were later immunized to the original antigen. This work was extended (Consden, Doble, Glynn, and Nind, 1971) and approximately 0.001% of the original amount of antigen injected intra-articularly in rabbits was shown to persist for up to 6 months.

The assumption that some at least of the antigen persisting in the normal rabbit knee may rest with the synovial membrane macrophages has been confirmed by Webb, Goldberg, Bluestone, and Pearson (1972), who were able to show that antigen can be identified on the surface of rabbit synovial fluid macrophages, these cells probably being shed directly from the synovial membrane into the joint cavity. The amounts of antigen persisting in the synovial fluid macrophages are similar to the very small amounts persisting in macrophages in other experimental systems.

This and other work discussing the role of macrophages in antigen handling and the passage of retained antigenic information by cell to cell contact to lymphocytes has been reviewed recently by Unanue (1972).

With this background, a search was made for the presence of macrophage-lymphocyte contact in the synovial fluid of patients with rheumatoid arthritis and allied conditions. Although most of the cells found in these synovial effusions are polymorphonuclear neutrophil leucocytes whose role appears to be to phagocytose complement-fixing immune complexes in the synovial fluid (see Zvaifler, 1973, for review), a proportion of the cells are macrophages, that is, mononuclear phagocytic cells. It was assumed that some of these macrophages, probably a small proportion, would be derived directly from the synovial membrane itself. It was also thought that these macrophages might bear on their surface an as yet unidentified primary antigen to which the patient's lymphocytes might be specifically sensitive. If so, it was anticipated that macrophage-lymphocyte clustering would be observed.

Materials and methods

MACROPHAGE PREPARATION

Synovial fluid was aspirated from the joints of six patients with rheumatoid arthritis. The fluid was defibrinated using glass beads and mixed with an equal volume of Hanks's balanced salt solution (HBSS). After centrifugation at 140 g for 10 min, the cell button was washed in Hanks's solution and resuspended.

Cell counts were then carried out. When more than 20% of macrophages were found, the cells were washed three times before being used. When a small percentage of macrophages was present, macrophage-rich populations were prepared by flotation on bovine serum albumin (Cline and Lehrer, 1968).

MACROPHAGE IDENTIFICATION

Macrophages were identified as large cells with abundant cytoplasm up to 25 μm in diameter, containing a single indented nucleus usually with two nucleoli. These cells adhered quickly to glass and migrated over the glass surface, rapidly taking up latex and colloidal carbon particles. These phagocytic cells also formed rosettes with human type O erythrocytes sensitized with anti-Rh O antibody, showing that they possessed surface receptors for IgG as has been shown previously for peripheral blood macrophages (Huber, Douglas, and Fudenberg, 1968).

MACROPHAGE CULTURE

Macrophages at a concentration of 1×10^6 cells/ml were suspended in medium 199 containing 10% fetal calf serum, penicillin 100 units/ml, and streptomycin 100 units/ml. The osmolality of this culture medium was 280 mOsm/l. The macrophages in their medium were cultured on glass coverslips in Sykes-Moore chambers at 37°C for one hour, to give the macrophages time to stick to the glass. The chambers were then gently emptied to get rid of nonadherent cells, dead cells, and debris. One chamber was then refilled with medium alone, another with lymphocytes from the patient's blood, and three further chambers filled with lymphocytes taken from healthy laboratory personnel who showed no evidence of arthritis. The concentration of lymphocytes in the medium used to refill the chambers was approximately 2×10^6 /ml.

LYMPHOCYTE PREPARATION

Peripheral blood was defibrinated with glass beads and centrifuged at 400 g for 15 min. The buffy coat cells were then removed and mixed with autologous serum and allowed to sediment for 4 hours in 1 g at 37°C (Weisbart, Webb, Bluestone, and Goldberg, 1972).

EXAMINATION OF CULTURES

These were examined from time to time by phase contrast microscopy and after 24 hours the coverslips were removed

from the chambers, gently washed in fresh culture medium, and fixed in methanol for Giemsa staining, or in 2% glutaraldehyde in medium 199 for scanning electron microscopy.

PREPARATION FOR THE SCANNING ELECTRON MICROSCOPE

After fixation in 2% glutaraldehyde for 30 min, coverslips were rinsed twice in Hanks's solution and post-fixed with 1% osmium tetroxide for 30 min, and rinsed twice in Hanks's solution. The coverslips were then dehydrated in graded alcohols for 10 min at each grade, and then from absolute alcohol through graded alcohol/amy acetate mixtures to absolute amy acetate. The coverslip was then transferred to a critical point drying apparatus (Boyde and Wood, 1969), taking care not to allow the amy acetate to evaporate before the apparatus was filled with liquid CO_2 . Gas was blown off until free from the smell of amy acetate when the chamber was sealed and heated gently, to bring the pressure in the chamber to 1500 lb/in². The gas was then gently released and the coverslip removed and coated in a vacuum chamber first with carbon and then with gold to provide a very thin conducting surface over the cells and coverslip. The prepared coverslip was then examined in a Cambridge stereoscan scanning electron microscope.

Results

Fig. 1 shows an example of an isolated macrophage seen under scanning electron microscope (SEM). The cell can be clearly seen migrating across the glass, with the spherical nucleus trailing behind. These appearances of the cell spreading on the glass surface, with an intricate series of folds in the membrane, are identical to the appearances in the SEM of isolated peritoneal macrophages (Warfel and Elberg, 1970).

FIG. 1 Synovial fluid macrophage. Scale 2 μm

Similar appearances were also identified in the living cell by phase and interference contrast microscopy.

Moreover, individual living cells were fixed in glutaraldehyde and re-examined afterwards, when no change in the appearance of the fixed cells could be seen. There is therefore every reason to suppose that the preparation of the cells for the SEM lead to very little or no distortion from the living state in culture. Clear evidence of clustering of spherical lymphocytes around macrophages was seen, as shown in Fig. 2. Such clustering could also be identified in methanol-fixed Giemsa stained prepara-

tions, but the clear identification of the spherical lymphocytes clustered on the macrophage surface was only seen under the SEM.

Isolated lymphocytes cultured on their own were also examined (Fig. 3) and shown to be spherical cells 3–4 μm in diameter. Recent work (Polliack, Lampen, Clarkson, De Harven, Bentwich, Siegal, and Kunkel, 1973), conducted after our study, suggests that the lymphocytes bearing villi are B or bursa-derived cells, while the smooth surfaced cells without villi are T or thymus-derived lymphocytes.

Fig. 4 shows two lymphocytes in contact with a

FIG. 2 Cluster of 5 lymphocytes around a synovial fluid macrophage. Scale 2 μm

FIG. 3 Two lymphocytes showing villi. Scale 2 μm

FIG. 4 Cluster of 2 lymphocytes about a macrophage. Scale 5 μ m

macrophage, where the lymphocytes are showing leaf-like villi on their surface. These lymphocyte-macrophage clusters were also seen in synovial fluid cells cultured after washing at 1 hour and without the addition of purified peripheral blood lymphocytes.

It was originally thought that only homologous lymphocytes would show such contact with synovial fluid macrophages. However, it rapidly became clear that the lymphocytes from the three normal donors would also undergo clustering with synovial fluid macrophages. This is presumably because in addition to the IgG receptors known to be present on macrophages, macrophages retain on their surface a large variety of different antigens in small amounts, to which the surface immunoglobulins of the B cells and also probably those less accessible immunoglobulins on the T cell surface, can make contact.

Therefore, although it is still possible that the synovial fluid macrophage may carry small amounts of some as yet undetected primary antigen which leads to specific contact with lymphocytes in and around the synovial cavity, the finding of clusters as shown here does not allow identification of these particular lymphocytes nor of the proposed primary antigen, while on the other hand the use of the SEM gives clear evidence of cell-cell contact which in conventionally stained preparations might be taken as chance overlying of one cell upon another during fixation.

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