Heberden Society

Clinical Meeting, September, 1970

At a meeting held at the Royal College of Physicians, Edinburgh, on September 25, 1970, the following papers were presented:

Adjuvant-induced Anti-red Blood Cell Activity in Mice: the Role of a Micro-organism isolated from Rheumatoid Joint Material. By ANN MCCrackEN and D. M. WEIR (*Immunology Unit, Department of Bacteriology, University of Edinburgh Medical School*)

An inbred strain of mice have been shown to exhibit a detectable immunological response to their own red blood cells on injecting either *Corynebacterium parvum* or SF 16—a bacterium isolated from the synovial fluid of a patient with rheumatoid arthritis. These micro-organisms have a marked adjuvant effect on the immunological system. The anti-red cell response has been detected by the immuno-cytoadherence test and the Jerne plaque method and animals injected with either organism show a pronounced increase in anti-red blood cell activity compared with saline-injected controls. This phenomenon has been correlated with haematological findings to determine the extent of any anaemia induced.

Doses of 0.1 to 8 mg. (dry weight) of heat-killed organisms were capable of producing the effect which was maintained for up to 3 weeks. It is proposed that the anti-red cell response may be due to stimulation of pre-existing antibody-forming cells and the antibody may act directly on red cells or facilitate their uptake by macrophages. This phenomenon, if it occurs in patients, may help to explain the anaemia of rheumatoid arthritis.

Discussion

DR. W. W. BUCHANAN (*Glasgow*) White and Gordon, as you pointed out, noted the adjuvant effect of diphtheroids obtained from a rheumatoid joint, and electron microscopy done on these organisms indicated that there were filaments on their surface similar to those they had seen in mycobacteria and in *Nocardia*, which of course have powerful adjuvant effects. I wonder whether you have examined your organism for this particular feature and whether you know of any organism that has an adjuvant effect but which does not have these peculiar filaments?

MRS. MCCrackEN I hope to carry out electron microscopy studies in the near future.

DR. T. M. CHALMERS (*Manchester*) Have you tried introducing any inhibitors such as corticosteroid preparations into this system?

MRS. MCCrackEN No.

PROF. E. G. L. BYWATERS (*London*) Is it possible that your bacteria have absorbed on to them something from the culture medium which might react with something coating the red cells?

MRS. MCCrackEN To the best of my ability I have excluded this. The bacteria were washed ten times after removal from the medium and when I injected some media there was no similar anti-red blood cell response.

Effect of Iron Dextran on Experimental Synovitis in the Guinea-pig. By A. G. MOWAT, T. F. DISNEY, and J. H. VAUGHAN (*University of Rochester School of Medicine and Dentistry, Rochester, N.Y.*)

This paper and the discussion thereon has been published in the *Annals* (see p. 201).

Radiological Assessment of the Cervical Spine in Rheumatoid Arthritis. By J. A. K. MEIKLE and M. WILKINSON (*Bridge of Earn Hospital, Perthshire*)

This paper and the discussion thereon has been published in the *Annals* (see p. 154).

Straingauge Plethysmography in the Assessment of Joint Inflammation. By I. VADASZ (*Guy's Hospital*)

This paper and the discussion thereon has been published in the *Annals* (see p. 211).

Study of Cells from Synovial Fluid in Tissue Culture. By J. M. K. MACKAY, W. R. M. ALEXANDER, and W. A. NEILL (*Rheumatic Diseases Unit, Northern General Hospital, Edinburgh*)

It is widely held that an abnormal immune response, probably of cell-mediated type, is present in rheumatoid arthritis. In view of the increasing evidence that macrophages have an important role in cell-mediated immune reactions a study of synovial fluid macrophages in tissue culture was undertaken.

Most previous work on synovial cells in tissue culture involved trypsinization, a procedure which would select against establishment of macrophage cultures. The method used in the present study was similar to that described by Lackington (1959). 76 cultures containing 20 per cent. heat-inactivated serum (from a single human pool), 50 per cent. whole synovial fluid, and 30 per cent. Eagle's medium were set up in Leighton tubes. Of 61 successful cultures, 47 were from patients with rheumatoid arthritis, twelve from patients with other inflammatory articular disease, and two from cases of primary generalized osteoarthritis.

The majority of the cells resembled those seen in cultures of peripheral blood from healthy donors. Multinucleate cells were present in varying numbers. All synovial cultures also contained large pale cells with abundant cytoplasm. 28 synovial cultures showed massive syncytial formations similar to those described by Palmer (1969).

Eight cultures grown on araldite and sandwich-embedded were examined by electron microscopy. This confirmed that a cell type, deficient in lysosomes and distinct from the characteristic macrophage, was present in synovial cultures. The nature of this cell has not yet been established.

References

- LACKINGTON, C. (1959) *Arthr. and Rheum.*, 2, 42 (Tissue culture of fibroblasts and round cells from arthritic synovial fluid).
PALMER, D. G. (1969) *Proc. Univ. Otago med. Sch.*, 47, 66 (Polykaryocytes and syncytial cell masses in dispersion cultures of rheumatoid arthritis).

Discussion

DR. A. J. PALFREY (*London*) Dr. Glen Bott for the last 3 years has been studying material from rabbit knee joints at St. Thomas's Hospital. She uses a trypsinization technique followed by culture and has studied both the washings and the cultures by electron microscopy. She has also studied the synovial membrane of the normal joint after this lavage technique and has shown that some of the surface cells of the synovial membrane have indeed disappeared as a result of that technique. The cells which she gets in culture, allowing for differences of electron microscopy technique, seem to me to be remarkably similar to those you are describing. She too has been plagued by crystalline-like structures, very similar in appearance to yours, which she attributed to the medium. As early as 4 hours after the start of the culture a large pale cell appeared with very few organelles. I wondered whether you had seen a comparable cell?

DR. MACKAY No. I think that the main feature of these cultures on electron microscopy is a cell which looks very much like a macrophage with its filopodia, but with a complete absence of lysosomes. These are large pale cells and appear very early in the life of the culture. In fact, as soon as the cells have spread, it is possible to distinguish them. I am very interested to hear about this work.

PROF. E. G. L. BYWATERS (*London*) When you examine synovial membrane from rheumatoid patients after synovectomy, you sometimes see that the whole of the surface is formed of columnar-type cells with very many multinucleate cells, rather like those you have shown.

My impression is that these are from knees which have been relatively fixed before surgery and have not had the opportunity of moving and perhaps disintegrating the surface by movement. Do you think that the multinucleate cells you have shown are related at all to either the A or the B type cells that have been described?

DR. MACKAY This is a very difficult question to answer because, in saying we believe this is like the reticular macrophage which Dr. Stuart has described, we have also to take into account the behaviour in terms of dye-uptake in culture. It would be very difficult to distinguish the cell in its situation *in vivo*.

Uptake of ^{14}C -Thymidine by Lymphocyte Cultures exposed to Human Synovial Fluid. By M. JEAN DAVEY (*Rheumatic Diseases Unit, Northern General Hospital, Edinburgh*)

Synovial fluids from thirteen patients with rheumatoid arthritis were tested as possible sources of antigen from altered tissues or infecting micro-organisms. Lymphocytes were separated from human blood by centrifugation through a Triosil-Ficoll mixture and microcultures were set up. The uptake of ^{14}C -thymidine was used as a measure of lymphocyte transformation.

In an autologous system, seven out of thirteen fluids caused slight to marked lymphocyte transformation at 6 days. Eleven of the thirteen synovial fluids were tested against autologous cells in the presence of phytohaemagglutinin (PHA) and nine of these caused enhancement of the PHA-induced response. Eleven synovial fluids were tested against homologous lymphocytes from rheumatoid patients: four of these caused slight transformation and five enhanced the lymphocyte response to PHA. In experiments with homologous lymphocytes from non-rheumatoid donors, four out of eleven synovial fluids caused a slight increase of ^{14}C -thymidine uptake, while six out of twelve enhanced the PHA response.

The increased uptake of ^{14}C -thymidine in lymphocyte cultures containing synovial fluid could have been due to:

- (1) Stimulation by an antigen to which the lymphocytes were sensitive;
- (2) Stimulation by anti-lymphocyte antibody;
- (3) Stimulation by lymphocyte mitogenic factor or other lymphokines;
- (4) Uptake of thymidine by micro-organisms replicating in the cultures;
- (5) Improvement of the lymphocyte culture conditions.

Discussion

DR. D. M. WEIR (*Edinburgh*) Regarding the possibility of this effect's being due to mitogenic factor, or to antibody, as you suggest, it should be feasible to test for this by fractionating the joint fluid on Sephadex G 200. You could separate out the MIF because it moves with the albumin fraction and the antibody would come out in one of the other fractions. Have you tried this?

DR. DAVEY Yes. We have carried out preliminary experiments using both Sephadex and DEAE fractionation, but we have had negative results so far.