Anti-DNA Antibodies in Discoid Lupus Erythematosus. By P. DAVIS, B. ATKINS, and G. R. V. HUGHES (*Department of Rheumatology, Royal Postgraduate Medical School*)

The exact relationship between discoid lupus erythematosus (DLE) and systemic lupus erythematosus (SLE) is still in some doubt. The high incidence of DLE in patients with SLE, the progression of between 1·3% to 5% of cases of DLE to develop SLE, and the identical histological nature of skin lesions in both diseases has led to the belief that DLE and SLE occur at two ends of a spectrum of the same disease (Dubois, 1966). Burch and Rowell (1971), however, have provided evidence to suggest that DLE and SLE are separate disease entities with different genetic background and natural history.

The aim of this paper was to study DNA antibodies in patients with SLE, in whom DLE was a feature and cases of cutaneous LE without systemic involvement. DNA antibodies have been shown to be highly specific for SLE and in particular have not been found in other diseases commonly associated with positive ANF's and LE cells (Hughes, 1971). Fifteen cases of DLE with systemic involvement, all of whom satisfied the A.R.A. criteria for the classification of SLE (Davis and others, 1973), 25 cases of DLE without evidence of multisystem involvement, and 20 normal controls have been examined. DNA antibodies were detected by the modified Farr technique. Results are expressed as a percentage. Normal range 0-30%.

Results Maximum DNA binding results have been recorded. All 15 cases with SLE had DNA binding above the upper limit of normal (range 39-93%; mean level 67%). All normal controls had DNA binding below 30% (range 0-27%; mean level 16%). In the patients with DLE there was a wider scatter of results (range 0-76%). Seven patients (26%) had DNA binding greater than 30% falling into the range usually associated with active systemic lupus erythematosus, although they had no systemic features. In these patients, the mean level 24% was statistically greater than in the normal group.

In view of the specificity of DNA antibodies for SLE it is suggested that these results support a relationship between discoid LE and SLE, and that they may have prognostic significance.

Discussion

PROF. E. G. L. BYWATERS (*Taplow*) It has been appreciated for a long time that these cases of discoid lupus who develop systemic manifestations tend to run very mild courses and have a very much better prognosis than those who are systemic from outset. I think this study begins to bear out this clinical impression.

DR. W. C. DICK (*Glasgow*) I may have missed this, but are you not really just trying to tell us that DNA antibodies are specific for LE on the one hand but not too specific on the other?

DR. DAVIS The clinical and serological similarities between these two diseases is well known. Our results confirm yet another similarity with the presence of DNA antibodies in both discoid LE and systemic LE. If you believe the hypothesis that these two conditions are pathogenetically similar, then our paper enhances the specificity of the test.

References

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Antigen Catabolism in New Zealand and Other Strains of Mice. By K. WHALEY, J. WEBB, and I. A. MORE (*Department of Pathology, Western Infirmary, Glasgow, and Centre for Rheumatic Diseases and University Department of Medicine, Royal Infirmary, Glasgow*)

The rates of catabolism of three soluble antigens, bovine γ -globulin, bovine serum albumin, and polyvinyl pyrrolidone have been studied in NZB, NZW, BWF1, BALB/c, CBA, and C3Hf mice. New Zealand mice catabolized these antigens more rapidly than nonautoimmune strains of mice. Experiments to investigate these findings have been performed and these include studies of thyroxine secretion rates and the effects of adjuvant administration on antigen catabolism. Although marked interstrain variations in thyroxine secretion rates were found they were not related to antigen catabolism. Injection of Freund's complete adjuvant to mice before antigen administration markedly increased catabolism rates in BALB/c and CBA mice, whereas NZB showed much smaller increases suggesting that the macrophages of NZB mice are already 'activated'. Preliminary electron microscope studies of splenic macrophages and Kupffer cells have shown increased phagocytic activity in NZB mice.

Discussion

DR. B. VERNON-ROBERTS (*London*) I think your results are very interesting, but there has been in recent years some stress on unaltered antigen which is retained on the cell membrane of the macrophage and it is this important component which induces the immune response and not the antigen that is broken down after being taken into macrophage. How important is catabolism?

DR. WHALEY I take your point. If we look at the rate of removal of aggregated protein antigens and aggregate free proteins, the rate of removal is possibly important in antibody production and resistance to tolerance. It is also known that in the rapidly removed part of the antigen the first part that sticks on the membrane is probably in the immunogenic part. All I would like to say is that I think these preliminary results are probably quite important. I think they may be more important in the resistance of immunological tolerance. In other words the faster an antigen is catabolized the less likely is its direct access to lymphocytes, if one believes this theory.

DR. D. C. DUMONDE (*Kennedy Institute*) It would be interesting to hear your present data on whether the half-life of serum proteins in these different mice is actually different. For example, if you injected labelled mouse albumin into these different strains of mice, whether you would find some differences. I think one of the interesting features about your results is that you seem to have established differences in different strains only with bovine γ -globulin as your foreign protein.

DR. WHALEY These results have been submitted to very stringent statistical analysis and the blood clearances of BSA and PVP are significantly faster in New Zealand mice than controls.

DR. D. C. DUMONDE (*Kennedy Institute*) Working with foreign species, globulin as antigen is something of a hazard for the experimental immunologist, because it may contain natural antibodies to certain mammalian tissue antigens. The question arises whether the results obtained with one such globulin antigen in various strains of mice are of general applicability.

DR. WHALEY I did mention this as a possibility. We have attempted to exclude this possibility by looking for natural antibodies and sucrose density gradient studies to see whether immune complex formation occurred. Furthermore, the more rapid rates of BSA and PVP catabolism in New Zealand mice suggest that natural immunity is not an important factor.

Studies on Synovial Fluid Lymphocytes in Rheumatoid Arthritis. By P. J. SHELDON, M. PAPAMICHAIL, and E. J. HOLBOROW (*M.R.C. Rheumatism Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berkshire*) *Annals*, 33, 509.

Heterogeneity of Human Synovial Tissue Cultures. By J. M. MARSH*, S. SPENCER, O. WIEBKIN, R. N. MAINI, D. C. DUMONDE, and H. MUIR. (*The Kennedy Institute of Rheumatology, London*).

Monolayer cultures from synovial explants have been reported to possess at least three morphologically distinct cell types: spindle, stellate, and multinucleate giant cells (Bartfeld, 1965; Smith and Hamerman, 1969; Smith, 1971; Castor and Dorstewitz, 1966). We report the characterization of the cell populations which are present in primary and long-term monolayer cultures by using phase contrast microscopy, a cell size analyser, and Ficoll density gradients.

Synovial tissue for culture was obtained during surgery, arthroscopy, or autopsy from twenty-five normal and thirty-seven rheumatoid joints; and for comparative purposes, twelve lines of skin fibroblasts were derived from skin excised at time of abdominal surgery. When cell growth from the explants became confluent, subcultures were obtained by disaggregating with trypsin and EDTA. All cultures were monitored for the presence of contaminating Mycoplasmas.

The morphology of cells using phase-contrast microscopy was studied in all cell cultures during the primary stage and an analysis of differences between normal and

Characteristic	Normal	Rheumatoid
Cell type	Spindle, stellate	Spindle, stellate
Nucleoli	Present	Increased
Nuclear budding	Occasional	Frequent
Multinucleate cells	Less common	Common
Particulate debris	Little	Increased
Contact inhibition	Present	Increased
	Overcome by 15-20% FCS	Overcome by 30% FCS
Cell proliferation rate		
0-72 hrs	No growth visible	Marked explant growth
1-2 wks	Slow	Rapid
2-6 wks	Rapid	Slow

rheumatoid cultures is documented, and in general agrees with the findings of other workers.

Secondary cultures (subcultures) were studied in nineteen lines of normal synovial tissue, seven of which were maintained up to 300 days in culture or fourteen passages. Twenty secondary rheumatoid cell lines were studied until the third to eighth passage. Only one rheumatoid cell line was maintained in continuous culture for 270 days. Normal synovial subcultures retained a more rapid population doubling time in comparison to the rheumatoid cell lines. After repeated passages, all cultures exhibited a tendency to become more homogeneous.

Using a cell size analyser, measurement of cell volumes during the first and second subcultures showed a non-sigmoid distribution indicating the presence of different cell populations. The results of measurements made on fifteen normal and four rheumatoid synovial cultures (at subculture 2) were analysed for their distribution by cell volume. Two or three distinct populations could be distinguished and their frequency in rheumatoid and normal cultures was determined. Preliminary results suggest that rheumatoid cultures have a preponderance of larger cells.

Ficoll gradients were used to separate the synovial cultures into at least two distinct populations. Changes in cell distribution on the density gradients after four or five passages have been observed in normal synovial cultures and may be ascribable to an ageing process.

The technique provides a basis for studying the behaviour of synovial cells *in vitro* and may be employed as an approach to the investigation of differences between rheumatoid and normal cells.

References

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Note

Revista Española de Reumatología

It is a pleasure to note the appearance of a new European journal on rheumatology—*Revista Española de Reumatología*. This is to be the official organ of the Spanish Society for Rheumatology, and takes the place of the previous *Revista Española de Reumatismo y Enfermedades Osteoarticulares*.

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