Reference Laboratory who provided us with the specimens of human and rabbit sera.

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Serum gamma-glutamyltransferase and alkaline phosphatase in rheumatoid arthritis

The paper by Spooner and colleagues¹ in the June issue prompts us to record our own observations on patients with rheumatoid arthritis. In a consecutive series of 46 patients (34 females, 12 males), all with positive serology, serum gamma-glutamyltransferase (GGT) activity was increased in eight patients (17%) and alkaline phosphatase (AP) in six (13%). This incidence of raised GGT is slightly lower than that found by Spooner *et al*, whereas the incidence of raised AP is much less.

On all patients showing raised serum enzyme activity we carried out isoenzyme examination by electrophoresis.^{2,3} GGT-2 was the principal GGT isoenzyme in six of the eight patients showing raised GGT activity, GGT-3 was present in all, and markedly increased in three. This pattern is typical of, but not exclusive to, patients with liver disease,⁴ and is especially found with intrahepatic cholestasis. In all six patients with increased total AP activity there was increased activity of the liver AP isoenzyme. In four of these, this was accompanied by increased "biliary" isoenzyme, and in one by additional increase of the bone isoenzyme.

Our studies indicate that in patients with rheumatoid arthritis showing raised activities of GGT or AP, this is likely to be of hepatic origin.

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Standardisation in the laboratory control of oral anticoagulant therapy

In collaboration with the International Committee for Standardization in Haematology (ICSH), the European Community Bureau of Reference (BCR) has produced three certified reference materials for the standardisation of commercial or laboratory-made human, bovine and rabbit thromboplastins, respectively. These reference materials have been calibrated against the WHO international reference preparation (IRP 67/40). By using the appropriate BCR reference material (human, bovine or rabbit) a sensitivity index can be assigned to any thromboplastin working preparation which will thus be directly related to the WHO primary reference preparation.

In clinical practice a prothrombin ratio obtained by means of thromboplastin reagent with an assigned sensitivity index can then be converted to an international normalised ratio (INR) by a simple equation: $INR = \text{antilog} (\log \text{prothrombin} \times \text{sensitivity index})$.

Manufacturers are being encouraged to establish the sensitivity indices of their thromboplastin reagents and to provide an appropriate Table of INRs. A therapeutic range for INR of 2.0-4.0 has been recommended.

Details of the scheme have recently been described.¹ Information of the availability of BCR Certified Reference Materials, a report of the certification protocol and recommended methodology for calibration of working preparations are available from

the European Community Bureau of Reference, Rue de la Loi 2000, Brussels B-1049, Belgium.

SM LEWIS

Chairman, International Committee
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Reference

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Direct evidence of localised immunological damage in vulvar lichen sclerosis et atrophicus

Lichen sclerosis et atrophicus (LSA) is known to be associated with an increased incidence of organ-specific autoantibodies¹ and autoimmune diseases². In addition a raised incidence of HLA-B40 in this disease has recently been reported and has led to the suggestion that this antigen may be in linkage disequilibrium with immune response genes controlling the susceptibility to both LSA and their autoimmune diseases³.

The present study has been designed to determine whether LSA is associated with immunological phenomena. A search has been undertaken for immunohistochemical evidence of deposition of immunoglobulin, complement (C3) and fibrin in the vulvar lesion and using adjacent normal skin as a control. In addition, sera from these patients have been screened for autoantibodies.

PATIENTS AND METHODS

Biopsies from 16 caucasian women (age range 36-78 yr, mean 64.6 yr) with vulvar LSA were collected over a period of six years. The histopathological diagnosis was confirmed by two independent observers and was based on the presence of hyperkeratosis, epidermal atrophy, homogenisation of the collagen of the upper dermis and an underlying chronic inflammatory cell infiltrate. Wedge biopsies of the affected and non-affected skin were snap-frozen and then examined by a standard direct immunofluorescence technique for the presence of immunoglobulin (IgG, IgA, IgM and IgE), complement (C3) and fibrin with commercially prepared fluorescein-labelled antisera (Wellcome Foundation and Hoechst Pharmaceuticals). Serum samples from 14 patients were obtained and screened routinely in the immunopathology laboratory for the presence of organ-specific and non-organ-specific antibodies

including thyroid, stomach and salivary gland and also for nuclear antibodies (ANA), smooth muscle antibodies and mitochondrial and reticulin antibodies. An indirect immunofluorescence method⁴ was used in the search for autoantibodies and, in addition, thyroid antibodies were sought using precipitin test, tanned red cell test (TRC, Wellcome Foundation) and later by the Thymune T and M tests (Wellcome Foundation).

RESULTS

Positive immunofluorescent staining was observed in 12 of 16 biopsies (75%), comprising fibrin (12 cases), C3 (4 cases) and IgM (4 cases). Two biopsies displayed positive staining for all three proteins. The staining was in relation to the epidermal basement membrane (Figure (a) and (b)) and, in *all* cases, only involved the histologically abnormal areas, the adjacent normal areas being devoid of staining.

Nine patients of 14 (64%) studied serologically had one or more autoantibodies (Table), but only five of 14 patients (36%) had organ-specific autoantibodies. Four patients (28%) had more than one antibody. Of the patients with matched serum samples and biopsies showing positive staining for fibrin, six of 12 (50%) had autoantibodies, and of those positive for IgM, C3, or both, three of five (60%) had autoantibodies. The three patients with positive serology but negative immunofluorescence all had non-organ-specific autoantibodies.

Using cryostat sections of normal skin and a direct immunofluorescence technique, no evidence of circulating antibodies to epidermal basement membrane was found in sera from six patients with LSA.

DISCUSSION

To our knowledge, this is the first report of immunoglobulin and complement (C3)

Incidence of organ-specific and non-organ-specific autoantibodies in patients (n=14) with LSA

Antibody	No. of patients with positive serology	%
Nuclear	3	21
Thyroid (thyroglobulin and microsomal)	5	36
Gastric parietal cell	1	7
Salivary gland	2	14
Reticulin	1	7
Mitochondrial	1	7
Smooth muscle	2	14

Indirect immunofluorescence technique showing linear deposition of (a) IgM and (b) C3 along the epidermal basement membrane of the affected vulvar skin $\times 375$.

deposition along the epidermal basement membrane. In earlier studies¹⁻³, LSA has been shown to be associated with an increased incidence of autoantibodies and a particular HLA antigen (HLA-B40) but the present findings offer direct support for the view that LSA has an autoimmune basis. In this study there is no evidence of

immunoglobulin and complement deposition in the basement membrane zone of the unaffected skin of patients with LSA, which contrasts with the findings in systemic lupus erythematosus⁵. Likewise in our study there are no detectable antibodies in the serum which react with the epidermal basement membrane of normal skin unlike SLE.

The overall incidence of organ-specific autoantibodies in our series of patients is similar to that reported in earlier studies,^{1 2} 36% as opposed to 44% and 32%, and our overall incidence of autoantibodies (64%) is comparable to that (74%) reported by Harrington and Dunsmore.² It is of interest to note that in our study, of the three patients with non-organ-specific autoantibodies, all had skin biopsies which displayed no immunofluorescence and this supports the idea that LSA is associated with organ-specific autoimmune disease.

The present findings suggest that vulvar LSA has an autoimmune basis. An obvious extension of this work would be a study of extragenital LSA in both sexes to determine whether this form of LSA has evolved in a similar manner.

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Top row: Almost total suppression of growth of control Oxford Staphylococcus (outer ring) and test strains (central inoculum) with, around the chloramphenicol disc, enhancement of growth in the area of subinhibition levels of this drug, of the control organism only (left) and both the test and control organism (right)

Bottom row: Near normal (left) and normal growth of the control staphylococcus (right) with severe inhibition of two different test strains (central inoculum) growth of that on the left being enhanced in crescentic fashion round the chloramphenicol disc (bottom) and the benzylpenicillin disc top

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Failure of growth on sensitivity testing agar

Over a period of three years there has been a recurring problem of failure of growth of organisms noted specifically on sensitivity plates containing lysed horse blood. The organisms most frequently involved have been wild strains of *Staphylococcus aureus* and occasionally *Staph epidermidis* but much less frequently Gram-negative rods including *Pseudomonas* have failed. The rarity of the phenomenon in relation to Gram-negative rods makes simple thymine

dependence unlikely and this is virtually excluded by the fact that occasionally the control organism is the only one failing to grow and sometimes both control and test are affected.

The phenomenon was first noticed on Isosensitest agar but occurs also on DST with lysed blood and on Isosensitest without lysed blood. Addition of supplements of thymine, thymidine and aneurin do not correct the problem. However on plates with a chloramphenicol disc, growth of the affected organisms is stimulated in a narrow band immediately at the edge of the zone of inhibition caused by the antibiotic—that is, in the zone of sub-inhibitory concentrations. Benzylpenicillin stimulates growth if the organism is resistant, but apparently not if the organism is sensitive.

We are quite unable to fathom the cause of this phenomenon. We are thus unable to reproduce the appearance in a model to test

any theories we may have. It does not appear to be related to the swab sticks used to inoculate the plates or the broths used to prepare the suspensions for inoculation, in which the organisms grow quite happily after subsequent overnight incubation. Have any of your readers encountered this phenomenon and do they have an explanation?

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