Letters to the Editor

PHOTOCHEMOTHERAPY FOR PSORIASIS

SIR,—Like Dr Rebora (June 6, p. 1258) we are not convinced by Dr Henseler and his colleagues that photochemotherapy with 8-methoxypsoralen and UVA (PUVA) does not maintain remission in psoriasis (April 18, p 853), indeed our own recent evidence is that it does. Since some of our earlier patients have been included in the European PUVA Study (EPS) upon which Henseler's conclusions were based an explanation is required.

The EPS concerns both the initial response of psoriasis (clearing) and prevention of subsequent attacks (maintenance). Our studies on clearance, although not cited in the report, have been in print for some time,[1,2] and our studies on maintenance were published recently.[3] Our findings on clearance were essentially the same as those in the EPS in which our patients were included. Thus the mean age of the patients, success rate, number of treatments required to clearing, and total cumulative dose of UVA were similar. However, in our study all patients, including those who went on to the maintenance part of the trial, had chronic plaque psoriasis whereas in the EPS 9·5% had guttate psoriasis which has a completely different natural history. The rash was much less extensive in our patients (more than 50% surface area affected in 4%) than in the EPS (more than 53% surface area affected in 50%). There was also a difference in our protocol in that we gave PUVA three times a week and most others in the EPS gave it four times a week.

In our own study, after clearance of the rash by PUVA, 112 patients were allocated at random to maintenance treatment once a week or once every three weeks and they were compared with a separate cohort who, after clearing with PUVA, received no maintenance. In the EPS several different maintenance regimens were used in an attempt to obtain the minimum effective maintenance dose for each individual. In our study patients were maintained on one of two fixed schedules irrespective of clinical state. It is to be expected, therefore, that the aim in the EPS study—to obtain fine adjustment of the PUVA maintenance dosage—will result in a higher relapse rate. Some patients continued treatment for 170 weeks whereas our results concern 64 weeks of follow-up. The EPS conclusion that relapse occurs as often with maintenance as without it is based on a definition of relapse ("New lesions or reappearance of old lesions which reduced the improvement rate by more than 15%") which is more complex than and different to ours ("Recurrence of psoriasis to involve 50% or more of body area that had been affected before the clearing regimen started"). Thus there are several factors which may explain our different conclusions about response to maintenance treatment. Furthermore at the time the EPS data was collected our part of the trial concerning maintenance was not sufficiently advanced for all our figures to be submitted for inclusion.

The EPS, collecting as it did data from eighteen centres, no doubt seemed a good idea at the time but we would doubt the significance of results obtained by treating different patients by different methods for different times and assessing them differently, specially since, with our data, only part was included in the analysis.

We do not agree that: "maintenance therapy may not significantly prevent recurrences for prolonged periods of time and may thus not be necessary in most patients". Our own evidence is that, up to 64 weeks, it certainly does have a clear beneficial effect. The difference between maintenance treatment once weekly and once every three weeks for this period of time was not significant, and treatment once every three weeks is therefore preferable. We calculated that in general patients would receive less UVA if they were given maintenance treatment once every three weeks than if they were allowed to relapse at the rate we showed they do relapse without maintenance and then given a further clearing course of PUVA. We agree that there are patients (about 20% in our series) who remain in satisfactory remission without maintenance but they cannot as yet be identified in advance. Our joint study was supported by the Medical Research Council and one objective of it was to determine the best regimens for the use of PUVA in the U.K. On our evidence, based on a carefully controlled and randomised study, clearance of psoriasis followed by maintenance treatment once every three weeks appears to be the best way of using PUVA. We believe that the contrary conclusion, that maintenance therapy is ineffective, is related more to the difficulties in executing and interpreting a multicentre trial than to the therapeutic reality.

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MONOCLONAL ANTI-MALIGNIN ANTIBODIES

SIR,—Malignin is a 10 000 molecular weight general transformation antigen of constant composition rich in glutamic and aspartic acids.[1-5] It is prepared from malignant glial cells grown in vitro. Polyclonal anti-malignin antibody from immunised animals or man has two components—one binds rapidly to malignin antigen (10 min) and one binds slowly (2 h). The polyclonal antibody produces specific immunofluorescence in a broad variety of malignant cells from human fluids and biopsy tissue and kills cancer cells in vitro at a concentration of approximately 1 ng of antibody protein per malignant cell. We now report the production of antibody-synthesising cell lines producing only the fast-binding species (monoclonal anti-malignin antibody-fast, MAMA-F) or only the slow-binding species (MAMA-S) or both (MAMA-FS). MAMA-F or MAMA-S alone bound to the antigen but did not destroy the cell, whereas a mixture of the two species or MAMA-FS labelled and destroyed malignant cells.

Myeloma cell line NS-1 was cultured in Dulbecco's minimum essential medium supplemented with 10% fetal bovine serum (D_{10}) in a humidified incubator at 37°C and 5% CO_2. Inbred female BALB/cJ mice, 8 weeks of age (Jackson Laboratory, Bar Harbor, Maine) were immunised with four weekly intraperitoneal injections of 20 μg malignin (Brain Research, New York) emulsified in complete Freund's adjuvant (Difco). Sera were tested for anti-malignin antibody by quantitative specific immunoadsorption to immobilised malignin, and antibody positive mice received one booster dose 4 days before cell fusion. Immune spleen cells (10^8) were fused with the myeloma cells (10^7) using polyethylene glycol[6] (PEG 1000, J. T. Baker). The PEG treated cell mix was seeded into 96 wells of a microtitre plate in D_{10} supplemented with hypoxanthine, aminopterin, and thymidine. Only the hybrid cells remained actively growing after 10 days. After two weeks in this medium, the hybrid cells were fed with medium D_{10} supplemented with hypoxanthine and thymidine for another week and then with D_{10} alone. Whenever the wells were about 80% covered by hybrid cells, supernatants were aspirated for anti-malignin antibody assay. Cells were identified by whether they produced one, the other, or both species (table). Cells from the antibody producing wells were cloned in soft agarose. The anti-malignin antibody positive clones were further grown intraperitoneally as ascitic tumours in BALB/cJ mice.

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3 Bogoch S, Bogoch ES. Production of two recognins related to malignin. Recognin M from mammary CF-7 carcinoma cells and recognin L from T. J lymphoma cells. *Neurochem Res* 1979, 4: 465–72.
4 Bogoch S, Bogoch ES. Tumor markers. Malignin and related recognins associated with malignancy rather than cell type. In Barbarin L, Hashim G, Lajtha A, eds. Neurochemistry and clinical neurology. New York: Alan R. Liss, 1980: 407–24.
5 Bogoch S, Bogoch ES. In Rosenberg SA, ed. Serologic analysis of human cancer antigens. New York: Academic Press, 1980: 693–96.

1 Vella Briffa D, Rogers S, Greaves MW, Marks J, Shuster S, Warin AP. A randomised controlled clinical trial comparing photochemotherapy with dithranol in the initial treatment of chronic plaque psoriasis. *Clin Exp Dermatol* 1978, 3: 339–47.
2 Rogers S, Greaves MW, Marks J, Shuster S, Vella Briffa D, Warin AP. Comparison of photochemotherapy and dithranol in the treatment of chronic plaque psoriasis. *Lancet* 1979, i: 455–56.
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ANTIBODY PRODUCTION MEAN (AND RANGE) FOR SETS OF CLONES

Antibody species	Antibody (μg/ml cell supernate)	
	At 1 mo	At 5 mo
MAMA-F	33 (19–67) (n = 14)	216 (126–393) (n = 6)
MAMA-S	33 (21–62) (n = 22)	107 (34–248) (n = 8)
MAMA-F/S*	27/25 (n = 7)	105/146 (n = 15)

*MAMA-F/S values in mouse ascites fluid at 8 months were, in two clones, 660/1070 and 780/670.

The table shows the quantities of monoclonal anti-malignin antibody produced by each antibody producing clone. The antibody yields increased by the fifth month of propagation. The cells continued to grow well through the eighth month and grew when transferred intraperitoneally to mice, where the yield of MAMA-S increased to as much as 1 mg/ml ascites fluid. The cells also grew well on soft agar.

All the three monoclonal antibody species stained malignant cells at concentrations of around 1 ng per cancer cell, specific immunofluorescence being obtained with human leukaemic blood, six cultured lines of leukaemia cells, three human lymphomas, and human malignant glial cells in vitro. Second layer fluorescent labels, both fluorescein and rhodamine, were active at concentrations as low as 1 in 1600, permitting elimination of non-specific staining. The stain was distributed on cytoplasmic and surface membranes while the nucleus was spared.

We have shown, in a study to be published elsewhere that polyclonal anti-malignin antibody is increased in the serum of patients with active cancer. In a seven year blind study of 1026 patients and controls in nine hospitals concentrations were 59±27 μg/ml in healthy controls and 273±157 μg/ml in patients with all types of active cancer. 83% of cancer patients with antibody levels below 135 μg/ml were dead within twelve months, whereas 90% of those alive up to four years had antibody levels above 135 μg/ml. Longitudinal studies demonstrated the drop in antibody before death. The availability of purified human polyclonal anti-malignin antibody and individual monoclonal antibody for the fast and the slow binding species permits exploration of the therapeutic possibilities of antibody acting alone or as a carrier for anti-cancer drugs.

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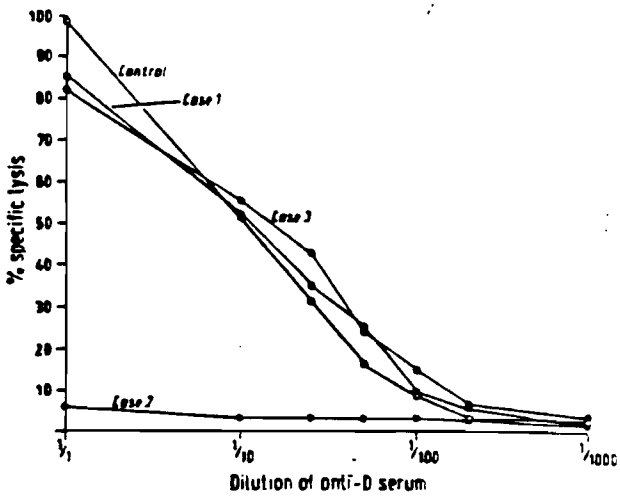
PREDICTION OF THE SEVERITY OF RHESUS HAEMOLYTIC DISEASE OF THE NEWBORN BY AN ADCC ASSAY

SIR,—In pregnancies where rhesus(D) hamolytic disease is suspected an indication of the prognosis can be had from the outcome of previous pregnancies[1] and from estimates of anti-D levels,[2] and optical density measurements on amniotic fluid may indicate when intrauterine transfusion should be considered.[3] We have previously noted variations in the potency of anti-D sera in



ADCC activity of anti-D sera from patients.

mediating (ADCC)[4] antibody dependent cell-mediated cytotoxicity and report here three cases where the ADCC (K-cell) assay predicted the outcome of haemolytic disease in the newborn (HDN) more accurately than did conventional assays.

Details of the three patients are summarised in the table. Representative sera were sent to Edinburgh for ADCC assay[5] without disclosing the clinical details. Sera were heat inactivated at 56°C for 30 min before testing. Comparison was made with a "standard" anti-D from a patient with a history of severe HDN, and all samples were tested on the same day with the same lymphocyte (effector cell) and red cell (target) donors. The results are expressed as % specific lysis, where the level of ^{51}Cr release from lysed red blood cells is corrected for spontaneous and maximum lysis of cells under the test conditions. Anti-D levels were measured[6] using the 1st British anti-D serum standard (72/229) as control

The ability of the anti-D sera to mediate ADCC of Rh(D) positive red blood cells is shown in the figure. Samples from cases 1 and 3 are highly active and result in lysis comparable to that of control anti-D while serum from case 2 was virtually inactive. The severity of HDN (table) correlated with these findings: cases 1 and 3 required exchange transfusion, whereas case 2 required only "top-up" transfusions for anaemia.

In these three cases the liquor ΔOD_{450} prediction of a favourable outcome was at variance with the anti-D levels which predicted poor outcome. There is a wide variation in the ability of anti-D sera from different sources to mediate lysis of Rh(D) positive red blood cells in an ADCC system;[4] and there is no obvious correlation between anti-D level (in IU/ml) and ADCC activity (as % specific lysis). Our findings indicate that assessment of the ADCC activity of anti-D sera gives better correlation with the clinical outcome than does quantitation by autoanalyser. The Liley test results were

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3 Liley AW. Errors in assessment of hemolytic disease from amniotic fluid. *Am J Obstet Gynecol* 1963; 86: 485–94
4 Urbaniak SJ, Greiss MA. ADCC (k-cell) lysis of human erythrocytes sensitized with rhesus alloantibodies. III. Comparison of IgG anti-D agglutinating and lytic (ADCC) activity and the role of IgG subclasses. *Br J Haematol* 1980; 46: 447–53
5 Urbaniak SJ. Lymphoid cell dependent (k-cell) lysis of human erythrocytes sensitised with rhesus alloantibodies. *Br J Haematol* 1976; 33: 409–19
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CLINICAL DETAILS AND LABORATORY FINDINGS

Case	History	Father	Anti-D (IU/ml)	ΔOD. . . (modified Liley)	Baby				Transfusions
					Group	Cord Hb (g/dl)	Cord Blood Bilirubin (μm/l)	Peak bilirubin (μm/l)	
Case 1 (A Cde/cde)	para 1 + 0, 1st affected pregnancy	O CDe/cde	25·8	Low zone	O pos. DAGT pos	11·0	80	330	1 exchange, 1 top-up
Case 2 (O cde/cde)	para 3 + 0, 2 previous HDN (mild)	O CDe/cDE	19·7	Low zone	O pos. DAGT pos	NA	20	107	2 top-up
Case 3 (O cde/cde)	para 1 + 1, no previous HDN	O CDe/CDe	49·7	Mid zone	O pos. DAGT pos	12·8	85	332	3 exchanges

NA = not available, DAGT = direct antiglobulin test, ΔOD. . . = optical density change at 450 nm, bilirubin units in μmol/l