**Introduction**

Attempts at immunological rehabilitation of patients with chronic mucocutaneous candidiasis (C.M.C.C.) include bone-marrow grafts, transplantation of thymus, administration of transfer factor, and injection of circulating lymphocytes. Clinical improvement has sometimes followed these treatments, but it has usually lasted only 6–12 months. In 1970 we described a female patient with severe C.M.C.C. who was unable to express delayed hypersensitivity to all antigens tested. Her lymphocytes were normally activated to DNA synthesis after stimulation with candida antigens in vitro, but this activation did not result in the release of detectable migration inhibitory factor (M.I.F.). We intended to treat this patient with HLA-identical lymphocytes from a healthy sibling, but she died from fulminating chest infection before this could be done. We describe here another patient with a similar clinical picture and the same type of immune defect who was given HLA-identical leucocytes from a healthy brother.

**Case-report**

**History**

The patient had severe candidiasis of the mouth and napkin area shortly after birth in 1959. By 11 months of age, his fingers became affected, resulting in severe nail deformity. There has been a chronic skin rash, blepharitis, and angulostomatitis from birth, and during the first 2 years of life he had recurrent boils. He had frequent bronchitic and febrile episodes with a hoarse voice and intermittent aphony. At the age of 7 extensive bronchiectasis was demonstrated, and he produced a copious of sputum daily. Primary smallpox vaccination at age 11 months resulted in twelve satellite blisters. There is no family history suggestive of immune deficiency.

**Physical Examination**

When seen in June, 1970, at age 12, the boy was between the 10th and the 3rd percentile for height and on the 3rd percentile for weight. There was a scaling dermatitis of his face, angulostomatitis, and oral lesions suggestive of chronic candida infections. There were no skin granulomas. The fingernails were dystrophic and there was a severe paronychia affecting the first and second fingers of both hands. Coarse crepitations were present over both lungs associated with a recurrent productive cough. The spleen could be felt 5 cm. below the costal margin.

**Investigations**

*Candida albicans* was repeatedly grown from sputum, skin, and nails. Routine investigations were normal except for a slight lymphopenia (900–1200 per c.mm.). No evidence of endocrine disorders was found and no auto-antibodies were detected in his serum. Serum IgA (68 mg. per 100 ml.) and IgM (37 mg. per 100 ml.) levels were normally reduced by these cells in vitro. Furthermore, in the same cells without stimulation. This ratio will be called the "index of replication activation" (I.R.A.).

**Materials and Methods**

**Lymphocyte Transformation**

We have slightly modified the M.I.F. assay method described earlier. Human M.I.F. was derived from lymphocytes, 5 × 10⁶ cells per ml., cultured for 3 days in medium 199 without serum and activated by candida antigens 100 µg. per ml. The supernatant medium was harvested daily and the cells resuspended in the original volume of fresh medium. No antigen was added after the first day. The supernatants from each culture were pooled, concentrated five times, and dialysed against 100 volumes of fresh medium 199. They were then filled with the test supernatants or culture medium 199. They were sterilised by filtration through 0-22 µ millipore membrane, and tested immediately or kept at −20°C and used within 2 weeks. Capillary tubes containing guineapig peritoneal exudate cells obtained by mineral-oil injection were sedimented by centrifuging at 450 g, cut, and placed into small circular chambers cut out of squares of silicone rubber and fixed by sterile silicone grease to an microscope slide. The chambers were then filled with the test supernatants or culture medium and sealed by mounting another microscope slide on the top, silicone grease again being used as a fixative.

In each experiment five sets of quadruplicates or quintuplicates, differing only in the composition of the fluid in the migration chambers, were set up as indicated below. All concentrated supernatants and culture media were enriched with 15% guineapig serum and their pH adjusted to 7-2 before use:

1. Fresh medium without antigen.
2. Fresh medium with 170 µg. candida antigens per ml.
3. Supernatant from unstimulated normal human lymphocytes, candida antigens being added after harvesting.
4. Supernatant from normal human candida-sensitive lymphocytes activated with the candida antigens.

**TABLE I—P.H.A.-INDUCED CYTOTOXICITY**

<table>
<thead>
<tr>
<th>Lymphocyte/target-cell ratio</th>
<th>Patient</th>
<th>Control I</th>
<th>Control II</th>
<th>Control III</th>
</tr>
</thead>
<tbody>
<tr>
<td>25/1</td>
<td>82%</td>
<td>80%</td>
<td>81%</td>
<td>81%</td>
</tr>
<tr>
<td>10/1</td>
<td>81%</td>
<td>82%</td>
<td>79%</td>
<td>Not done</td>
</tr>
<tr>
<td>1/1</td>
<td>84%</td>
<td>61%</td>
<td>68%</td>
<td>50%</td>
</tr>
</tbody>
</table>

Target-cell damage was determined by measuring the release of chromium-51 into the culture medium. The results are expressed as the percentage of radioactivity in supernatants from target cells suspended in distilled water.
(5) Supernatant from the patient's lymphocytes activated with the candida antigens.

System 2 indicated any macrophage preparation contaminated by candida-reactive guineapig lymphocytes, and such an assay would be discarded. System 3 (usually

showing slightly more migration than systems 1 or 2) was used as the 100% reference area. The area of migration was measured at 24 hours and expressed as a percentage of the reference area (M.I.F. index).

Cytotoxicity Assay

P.H.A.-inducible cytotoxicity was tested by using a modification of the method described by Holm and Perlmann. The effector cells were separated from defibrinated blood by centrifugation on a 'Triosil-Ficol' gradient. The leucocyte pellet consisted of 80-95% lymphocytes, 5-17% monocytes, and 1-3% polymorphonuclear cells. More than 98% of the cells remained viable. Chicken erythrocytes were used as target cells. They were derived from heparinised blood and labelled with chromium-51. Contamination with polymorphonuclear cells up to 20% did not influence the chromium release in our experiments. The experiments were set up in duplicate and to each tube was added 10⁵ target cells. Lymphocyte/target-cell ratios of 25/1, 10/1, and 1/1 were used, and the cell mixtures were suspended in a total volume of 1-5 ml. of medium 199 containing 5% heat-inactivated fetal calf serum. Each experiment included the following combinations: chicken erythrocytes alone (a) or with P.H.A. (b), lymphocytes (c), or lymphocytes plus P.H.A. (d). The tubes were incubated for 24 hours under the same conditions as for the transformation experiments. After incubation the tubes were centrifuged at 350 g for 5 minutes. 1 ml. of the supernatant of each tube was carefully aspirated and its radioactivity was determined. The radioactivity in the supernatant of each tube was used as a measure of cell damage. The results were expressed as the percentage of the activity in supernatants from target cells, which were suspended in 1-5 ml. of distilled water 24 hours previously.

Leucocyte Transfer

The donor was the patient's 4-year-old brother. They were both of HL-A type 2, W19, W12 (Dr. S. Lawler), and their lymphocytes were not activated in a mixed culture. The donor is healthy and had strong delayed skin reaction to candida. His lymphocytes were able to release M.I.F. activity in vitro (M.I.F. index 62). He was bled, 100 ml. at a time, into plastic bags (Fenwal) containing acid citrate dextrose. The red blood-cells were sedimented with the aid of dextran and returned to the donor, but the white blood-cells were immediately transferred to the patient, who received a total of 3 × 10⁹ mononuclear leucocytes (10⁸ cells per kg. body-weight). This was done in August and October, 1970.

Results

The patient's lymphocytes were rather more effec-

![Fig. 1-The patient before (a) and 16 months after (b) the first leucocyte transfusion.](image)

![Fig. 2-Height and weight percentile charts.](image)
tive in lysing target cells after P.H.A. stimulation than were lymphocytes of three healthy controls tested simultaneously (Table I). His lymphocytes were also more reactive in lysing target cells after P.H.A. stimulation than activated to D.N.A. synthesis after stimulation with candida antigens, and D.N.C.B. sensitisation failed twice (Table II).

After the leucocyte transfusion the patient has made good steady progress. He has attended school every day during the past year. In November, 1971, he came second in the cross-country run at his school. His skin and mucous membranes are now normal, his lungs have cleared, and he now only rarely produces sputum. Two of his nails are still dystrophic, however, and his spleen has not got smaller. Fig. 1 shows his face before and after the treatment, and Fig. 2 demonstrates his physical development. Table II summarises the immunological progress. He can now express delayed hypersensitivity to P.P.D. or candida, and D.N.C.B. sensitisation failed twice (Table II). The patient was unable to detectable M.I.F. activity. The patient was last seen in December, 1971, when his clinical and immunological normality has ensued. This activation did not, however, result in the generation of a detectable M.I.F. activity. The patient was unable to express delayed hypersensitivity to P.P.D. or candida antigens, and D.N.C.B. sensitisation failed twice (Table II).

Discussion

We believe this is the first time that HL-A identical blood leucocytes from a sibling have been used in the treatment of c.M.C.C. Gradual restoration towards clinical and immunological normality has ensued. This could be due to release of transfer factor from the donated cells or to the grafted cells remaining functionally competent in the recipient. Transfer factor probably acts primarily on the afferent part of the immune response, which was presumably intact in this patient since his lymphocytes could be activated to D.N.A. synthesis with candida antigens in vitro.

Another of our patients with the same type of immunological defect had received 10 units of blood without any clinical or immunological improvement. We, therefore, consider it unlikely that the improvement in this patient can be attributed to transfer factor alone—indeed, when lymphocyte extract has been given to patients with c.M.C.C. improvement, if it takes place, has usually only lasted 3–8 months, after which a further injection is required. Furthermore, the fact that the patient has now been successfully sensitised to D.N.C.B. after two unsuccessful attempts before the lymphocytes were injected argues in favour of a functioning graft. The donor had not been sensitised to D.N.C.B. and attempts to transfer D.N.C.B. contact sensitivity in man with dialysates of peripheral leucocytes have not been successful. The transfused lymphocytes are, therefore, presumably supplying a population of cells capable of producing delayed hypersensitivity. Recent studies on circulating leucocytes indicate that a small proportion of these cells have stem-cell properties and can clone in vitro. The striking effects of the relatively few lymphocytes that our patient received may be attributable to cloning in vivo.

Preliminary Communication

STEREOTACTIC LESIONS IN THE KNEE OF THE CORPUS CALLOSUM IN THE TREATMENT OF EMOTIONAL DISORDERS

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Summary

Electrical high-frequency stimulation of the genu of the corpus callosum (g.c.c.) immediately abolished anxiety and tension in psychiatric patients with these symptoms. Simultaneously, the patients experienced a feeling of inner wellbeing and relaxation. Subsequent permanent surgical lesions corresponding to the site of emotional response to stimulation, 6 x 6 to 8 x 12 mm. in diameter, seemed to relieve the symptoms. Similar stimulation of the g.c.c. of patients with involutional melancholia did not evoke subjective responses, nor did the corresponding surgical lesions seem to be effective. The findings are based on stereotactic treatment of eleven patients with intractable anxiety, fears, and tension and three patients with involutional melancholia. It is assumed that in anxiety and tension the interhemispherical transcallosal cingulostriate pathways are hyperactive.

INTRODUCTION

Until recently I had produced small stereotactic lesions in the rostral cingulum, anteroventrally to the genu of the corpus callosum (g.c.c.) in psychiatric patients with intractable anxiety of varied origin. This target had been selected because at this point the cingulum is easy to visualise radiographically and so narrow that a small lesion interrupts the pathway completely. In 25 of the forty-six patients treated bilateral lesions varying in size from a pea to a coffee