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Expression of Blood Group Antigens by Cultured Human Epidermal Cells Used as Allografts

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The expression of blood-group antigens was studied on human epidermal cultures used as allografts in 13 non-immunosuppressed patients treated for leg ulcers. The study was carried out using monoclonal antibodies to A and B antigens by an indirect immunofluorescence technique. Blood-group antigens are weakly expressed on the suprabasal layers of the cultured epidermal sheets. After grafting, the donor's blood-group antigens were detected on a few cells of the suprabasal layers. Furthermore, scattered keratinocytes as well as acrosyringia were found to express the recipient's blood-group antigens. (Received August 5, 1986.)

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Blood group antigens A, B and H are glycolipids and glycoproteins found on erythrocytes, some body secretions and tissue (1, 2, 3). They have been detected by immunofluorescence in normal adult stratified epidermis, oral mucosa and also in neonatal skin, and human epidermal cultures (4).

Human epidermal cultures were used in allogeneic patients without any clinical or histological sign of rejection (5, 6, 7), presumably thanks to the absence of Langerhans' cells from the grafts (8, 9, 10, 11).

In this work, we investigated the expression of blood-group antigens by an indirect immunofluorescence (IIF) method using monoclonal antibodies (mabs) in patients grafted with allogeneic epidermal cultures.

MATERIAL AND METHODS

Human cultured epidermis was prepared according to the method previously described by Howard Green (Fig. 1) (12). Confluent secondary cultures were detached from the tissue-culture dish using Dispase II and then placed on a vaseline gauze with surgical clips to be grafted (6, 7).

Patients

Thirteen non-immunosuppressed patients suffering from chronic leg ulcers (recipients) were grafted with skin taken on the anterior face of the forearm. The epidermal allograft was placed on the graft-taking area the same day. Blood-group typing was performed in every pair (recipient/donor) before grafting. Punch biopsies were taken from the grafted site from day 5 to day 150, and kept frozen until used for indirect immunofluorescence studies.

Indirect immunofluorescence studies

Frozen sections (4 μm) were cut, air-dried and fixed in acetone for 10 min at 4°C. Immunofluorescent staining was performed as follows: sections were incubated with first-layer anti-sera, i.e. monoclonal antibodies to blood group substances A and B (CNTS, Paris, France) (dilution 1 : 10) for 45 min at 37°C and washed in PBS for 25 min. Fluorescein conjugated second-layer anti-serum (goat antimouse IgM labelled with FITC, dilution 1 : 20 (TAGO, Inc. Burlingame, Ca) was added, incubated and washed as above.

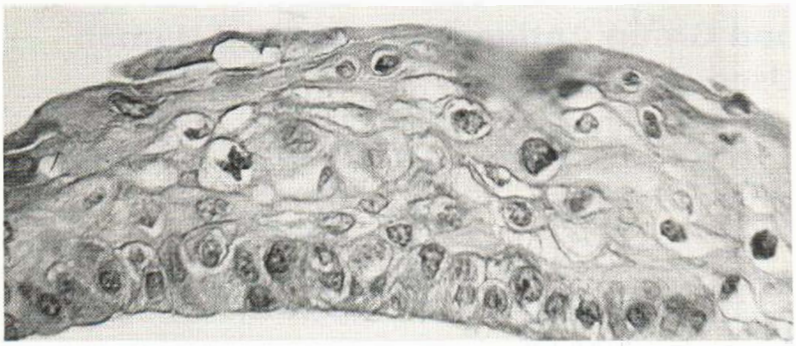

Fig. 1.. Cultured epidermal sheet consisting of a basal cell layer and 5 to 7 supra-basal layers (H and E, x350).

Controls were carried out with normal mouse serum + FITC conjugated anti-mouse IgM, and PBS + FITC conjugated anti-mouse IgM, and were consistently negative. Slides were mounted in a polyvinyl-alcohol mounting medium and examined under a ZEISS fluorescence microscope with epi-illumination.

RESULTS

On normal skin, A and B antigens in patients of the respective blood group were found in the stratum spinosum and stratum granulosum but not in the basal cell layer and the stratum corneum. These antigens were also expressed by erythrocytes and vascular endothelium in the dermis (Fig. 2).

Fig. 2. Expression of blood group antigens by normal skin. Dermal vessels, spinous and granular layers express these antigens. IIF with monoclonal antibodies to substance A (x220).

Table I. *Expression of blood group antigens by human epidermal cultures*
mabs = monoclonal antibodies, – = no reaction, + = weakly positive

Human epidermal cultures		Immunofluorescence: Mabs to blood group substances	
Day of secondary culture	Blood group	A	B
13	0	–	–
10	A_1	+	–
41	0	–	–
17	0	–	–
10	0	–	–
17	A_2	+	–
14	A_1	+	–

Table II. *Expression of blood group antigens by allografted human epidermal cultures*
mabs = monoclonal antibodies, – = no reaction, + = weakly positive, ++ = moderately positive

Recipient		Epidermal culture grafted	Day after grafting	Immunofluorescence with mabs to blood group substances	
				A	B
1	A_1	A_2	15	+	–
			28	–	–
			42	–	–
2	A_1 B	B	15	–	+
			14	–	+
3	0	A_1	30	–	–
4	0	A_1	5	–	–
			14	+	–
			28	–	–
5	0	A_1	5	–	–
			14	+	–
			28	–	–
6	0	A_1	14	–	–
			20	–	–
7	0	A_1	21	+	–
			28	+	–
8	B	0	14	–	–
			28	–	+
9	A	0	7	+	–
			30	+	–
10	A	0	10	–	–
11	A_1	0	15	+	–
			28	+	–
			48	+	–
			150	++	–
12	0	0	14	–	–
			28	–	–
			45	–	–
13	0	0	12	–	–

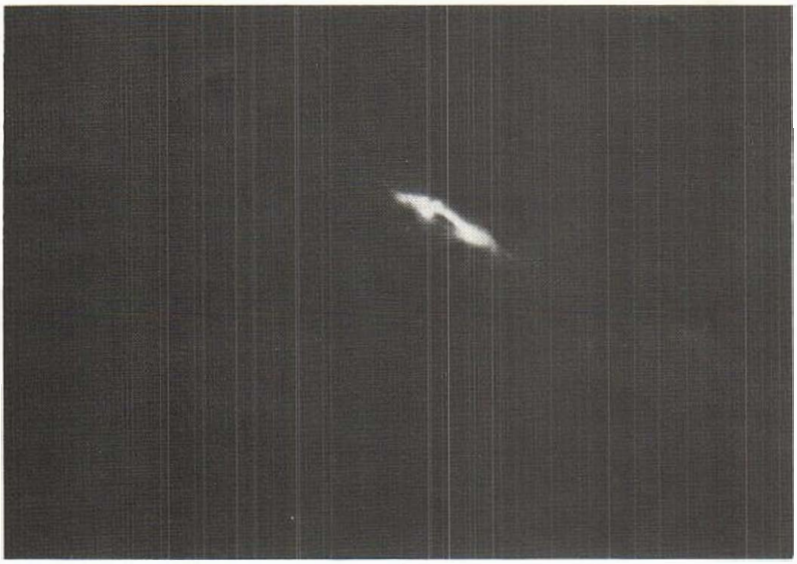

Fig. 3. Expression of blood group antigens by a human epidermal culture from a donor A. A few supra basal cells are stained. IIF with a monoclonal antibody to substance A (x350).

Human epidermal cultures (Table I)

The expression of blood group antigens was weak on the epidermal sheets taken at various intervals of time (n=7). A few supra-basal cells were positive when the donor had the blood group A, i.e. in 3 cases (Fig. 3). We did not get any epidermal culture from donor B or AB for this part of the work. None of the epidermal cultures from donor O was positive. Lastly, in our experience pre-treatment of the sections with trypsine did not increase the staining intensity.

Epidermal allografts (Table II)

In all cases the blood group antigen of the recipient (either A or B) was expressed by the endothelial cells of the vessels in the dermis. Concerning the expression of blood group

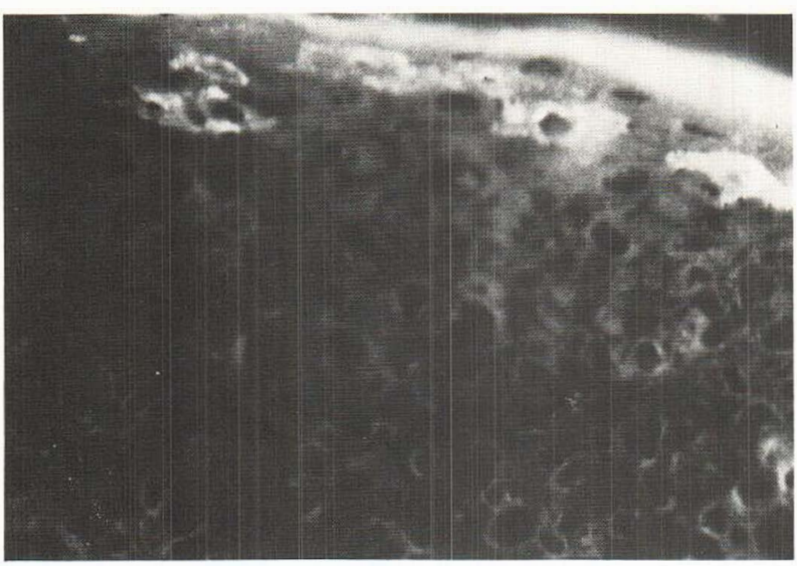

Fig. 4. Human epidermal culture from a donor A grafted on a recipient O. On a biopsy taken at day 21 a few positive cells in the grafted epidermis are seen. IIF with a monoclonal antibody to substance A (x220).

Fig. 5. Human epidermal culture from a donor O grafted on an allogeneic recipient A. On a biopsy taken at day 28 no positive cell is found in the epidermis, but the vessels express the blood group antigens of the recipient. IIF with a monoclonal antibody to substance A (x220).

substances by the grafted epidermis different situations have to be discussed separately:

Recipient A/donor A (patient 1): Blood-group substances A were found only on the first biopsy—at day 15—in this patient.

Recipient AB/donor B (patient 2): Only the blood-group substance B was found into the upper layers of the grafted epidermis.

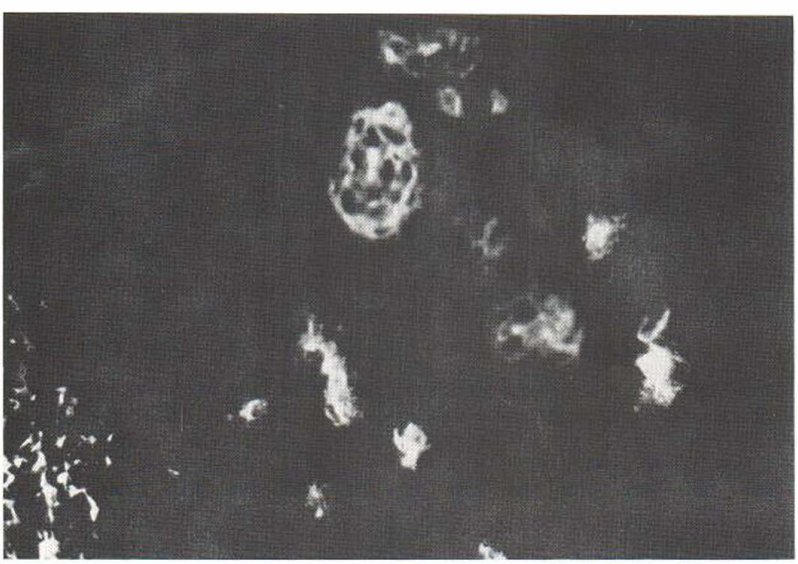

Fig. 6. Human epidermal culture from a donor O grafted in a recipient A. On a biopsy taken at day 14 an acrosyringium expresses blood group A in the grafted epidermis O. IIF with a monoclonal antibody to substance A (x220).

Recipient O/donor A (patients 3, 4, 5, 6, 7): In 4 patients out of 5, blood group antigen A was found weakly positive up to day 28 in the spinous or the granular layer of the grafted epidermis. In one patient (no. 3) no blood-group antigen could be detected on a biopsy taken at day 30.

Recipient A or B/donor O (patients 8, 9, 10, 11): A few cells expressing the blood group antigens of the recipient (i.e. in patient 9; A in patients 9, 10 and 11) were found into the upper layers of the epidermis (Fig. 5). In patient 11, more cells were stained on the last biopsy taken at day 150. Structures into the grafted epidermis identified as acrosyringia expressed strongly the blood-group antigens of the recipient after day 15 (Fig. 6).

DISCUSSION

In this work we studied the expression of blood-group antigens on epidermal cell cultures used as allografts in 13 non-immunosuppressed patients without any clinical or histological sign of rejection during a 12 months' follow-up. A and B antigens are present in the spinous and granular layers of the normal epidermis but are not expressed by the basal cell layer (13, 14, 15, 16). We found that the expression of human blood-group antigens in epidermal cells grown in vitro (12) is restricted to a few supra-basal cells. Thompson et al. (4) found a strong expression of blood-group antigens by cultured human epidermis. This discrepancy may be related to the culture procedures, since these authors used an explant method.

Trypsinization of the preparations was not necessary for the demonstration of the blood group antigens in the epidermis. As compared to the work of Dabelsteen (15) this may be explained by the differing properties of the antibodies used.

Other studies from our group showed that some antigens expressed in normal epidermis are not found or only weakly present in human cultured epidermis: HLA class I antigens are found only on the basal cell layer, and are progressively expressed on the suprabasal layers after grafting (17). Desmosome associated antigen (KM48) is not found on cultured epidermis but is present after grafting (18).

The weak expression of blood group antigens in human cultured epidermis may be related to an incomplete differenciation in vitro. In vivo, in some neoplasms originating from epithelium in which blood substances are normally present; a partial or even a complete loss of these antigens has also been reported (19).

After grafting, the presence into the epidermis of cells expressing blood group substances of the donor demonstrate the identity of the surface epidermis to the culture grafted.

We also identified isolated cells expressing the blood group antigens of the recipient into the upper layer of the grafted area. These cells, possibly are of adnexal derivation since acrosyringia are reconstituted into the epidermis grafted.

This study indicates that the expression of A and B blood group substances by human keratinocytes is weak in vitro and this factor does not seem to be able to induce any rejection of the cultured epidermis used as allograft (4).

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