Transfer of the White Graft Reaction.* (32521)

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Skin grafts from allogeneic donors are rejected by specifically sensitized mice in one of three modes(1): a) prior to destruction of graft, its surface and follicular epithelium undergoes hyperplasia, and a heavy lymphocytic infiltrate is seen; this process is essentially an acceleration of the rejection process seen in non-sensitized mice, and has been designated as "blue" because of its distinctive histological appearance; b) the surface and follicular epithelium of the graft undergoes early degeneration and necrosis, lymphocytes are scanty but tend to accumulate at the host-graft interface, the blood vessels are markedly dilated, and hemorrhage is widespread; this type of graft has been designated as "red"; c) as in "red" grafts, there is early epithelial necrosis and scarcity of infiltrating lymphocytes, but the graft fails to become vascularized; this type of graft was designated several years ago as "white" by Taylor *et al*(2) and Rapaport *et al*(3).

Investigators agree that the white graft reaction in man and laboratory animals is the expression of a particularly intense, relatively short-lived immune response demonstrable early after onset of sensitization. However, the precise mechanism of this reaction is not understood. The mechanisms of other types of graft rejection have been clarified by transfer studies. Transfer of second-set reactivity is usually accomplished readily with sensitized lymphoid cells (in man, also with cell extracts), while serum transfer has often been unsuccessful. Hence, investigators tend to believe that graft rejection is brought about by cell bound mechanisms, and not by serum antibodies. However, several investigators have considered the possibility that the white graft reaction is an exception. Lawrence *et al* (4) were unable to transfer second-set reactivity with cell extracts when the donors of the cells were in a state of white graft reactivity. They therefore considered the possibility that white graft reactivity was not associated with cell bound antibody. Several years ago, Stetson *et al*(5) presented data indicating that the white graft reaction could be transferred by means of serum. Also, very violent rejections of homografted human kidneys are occasionally referred to as being of the "serum type." However, recent data by Perey *et al*(6) indicate that chickens rendered agammaglobulinemic by bursectomy and irradiation are not only able to reject skin grafts in a normal fashion, but are also able to show a white graft reaction; this implies that, in birds, the white graft reaction is not a serum-mediated effect.

The data to be presented concern attempts to transfer the white graft reaction by means of serum and of lymph node cell suspensions.

Materials and methods. The donors of serum or sensitized lymph node cells were BALB/c mice sensitized with skin grafts from C57BL/6 or C57BL/10 mice. The recipients were of the BALB/c strain, and were tested with C57BL skin grafts, the grafts being studied microscopically 6 days after transplantation. All mice were obtained from our own colonies. Serum or lymph node cell suspensions were transferred 6, 8, 10, or 16 days after onset of sensitization; when donors were sacrificed on the 16th day, a second sensitizing skin graft was applied 10 days after the first. Serum was injected intravenously, in doses of 0.4 to 0.8 ml, and usually the day after test grafting. In one experiment, 3, and in another 4 successive daily doses of serum were given; in the former, injection began the day before, and in the latter 2 days before grafting. Cells, obtained by mincing the ipsolateral axillary and brachial lymph nodes in Hank's solution, were injected intraperitoneally, in doses of 1 to 210 million, usually as single injections. They were administered over a broad time range, from 5 days before to 3 days after test grafting. In several experiments, successive daily doses were administered, following schedules described below. The dose and time

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scheduled were chosen to obtain maximal effect following preliminary studies, the schedules in general corresponding to those used by Stetson *et al*(5); also, Billingham *et al*(7) were most successful in transferring sensitivity when the cell donors had been sensitized 8 or 11 days previously.

Results. None of 54 recipients of *serum* showed a white graft reaction, nor was there unequivocal evidence of other forms of accelerated rejection.

Cell transfers were more effective (Table I). There was evidence of accelerated rejection in 110/164 mice. Following the transfer of 1 to 10 million cells, less than half of the recipients showed signs of sensitization, while all recipients receiving 210 million cells were sensitized. It made little difference whether the sensitized cells were injected 3, 2, or 1 day before grafting, or 1 to 2 days afterwards. Transfer was ineffective if performed 5 days before or 3 days after grafting. When cell donors were sacrificed 16 days after sensitization, transfers were essentially ineffective, as were transfers performed after only a 6 day sensitization period. The intravenous route was less effective than the intraperitoneal route. Injection of cells around the graft bed likewise had little effect, suggesting that the injected sensitized cells did not have ready direct access to the graft bed.

TABLE I. Effect of Transfer of Lymph Node Cells* from Sensitized† BALB/c Mice on Reactivity to C57BL Skin Grafts.

Time of transfer‡ (days)	No. mice	No. with white grafts	No. with accel. rej. other than white grafts	No. without evidence of sensitization
−5	5	0	1	4
−3	9	0	8	1
−2	33	0	18	15
−1	11	0	8	3
0	49	2	31	16
+1	10	0	6	4
+2	14	1	5	8
+3	10	0	0	10
−2, −1, 0	13	2	11	0
−3, −2, −1, 0, +1	10	9	1	0

* No. of transferred cells: see text.
† Cell donors sensitized by C57BL skin grafts 8 or 10 days previously: see text.
‡ −: cells transferred prior to grafting; +: after grafting.

White grafts were only rarely seen after single transfers (3/147): in 1 of 14 mice receiving 16.5 million cells (2 days after grafting), and in 2 of 40 mice receiving 20 million cells (on the day of grafting). None of 4 mice receiving 210 million cells (2 days before grafting) showed a white graft. However, 2 of 7 mice receiving injections (12 to 85 million cells per injection) on 3 successive days (starting 2 days before grafting) showed white grafts. Because the incidence of white grafts was relatively high following multiple transfers, a final group of 10 mice received 5 successive daily doses (20 million cells per injection) starting 3 days before grafting. Nine of these 10 mice showed white grafts, and the remaining mouse also was sensitized.

Summary. White graft reactivity in mice could be transferred by sensitized lymph node cells provided transfers were performed repeatedly, and over a sufficiently long time. Serum transfer was universally unsuccessful. These observations support the belief that the white graft reaction is brought about by a cell bound mechanism and that its pathway does not differ qualitatively from that utilized in other types of graft rejection.

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