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Vascular Endothelial Cells in Cell-Mediated Immunity: Adoptive Transfer With In Vitro Conditioned Cells Is Genetically Restricted at the Endothelial Cell Barrier

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Delayed-type hypersensitivity (DTH) is a cell-mediated immune response that can be adoptively transferred in rats when greater than 2×10^8 cells from peritoneal exudate, lymph nodes, or spleen are used. We have shown that by using an in vitro conditioning step with antigen, transfer can be subsequently carried out with as few as 2×10^7 spleen cells. The magnitude of DTH was reflected in ear swelling after intradermal injection of antigen [tuberculin or keyhole limpet hemocyanin (KLH)] and confirmed histologically. The transfer was antigen specific, requiring the sensitizing antigen in both the in vitro conditioning step and in the ear test challenge. Adoptive transfer with conditioned cells was genetically restricted by alleles of the RT-1 region [major histocompatibility complex (MHC) of the rat]. Brown Norway strain (n haplotype) immune cells would not transfer DTH to Lewis (l haplotype), ACI (a haplotype), or Buffalo (b haplotype) rats, whereas each strain would transfer DTH to syngeneic recipients. Moreover, this pattern of restriction held for all strains when tested in reciprocal fashion. In additional experiments, F_1 to parental bone marrow chimeras were constructed so that bone-marrow-derived cells and non-bone-marrow-derived cells were of different RT-1 haplotypes. When these chimeras were used as recipients, transfer of DTH was only observed when immune donor cells and recipient non-bone-marrow-derived cells were syngeneic. These results point to the critical role of non-bone-marrow-derived cells (endothelial cells) in the DTH reaction.

Key words: endothelial cells, cellular immunity, adoptive transfer, antigen presentation

Delayed-type hypersensitivity (DTH) is defined as a cell-mediated immune response because of its adoptive transfer characteristics with T lymphocytes. Lefford et al [1] have shown that thoracic duct lymphocytes from rats immunized with bacillus

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calmette guerin (BCG) are capable of transferring DTH to purified protein derivative (PPD) of tuberculin in naive syngeneic recipients. The magnitude of DTH transferred and the antitubercular immunity conferred in these animals were dose-dependent, with more lymphocytes required to transfer DTH than immunity. A 24-hr collection of thoracic duct lymph was required to obtain 5×10^8 cells, the minimum number necessary for DTH transfer.

Experience with the experimental allergic encephalomyelitis (EAE) system has shown that adoptive transfer of EAE was also possible, but only with high numbers of cells collected in a narrow interval prior to the development of clinical disease. Moreover, as many as 10^9 spleen cells from animals that had recovered from EAE did not transfer disease. However, after an in vitro conditioning step with concanavalin A [2] or myelin basic protein (MBP) [3], adoptive transfer to syngeneic recipients was possible with as few as $1–2 \times 10^7$ cells. This in vitro conditioning step has subsequently been used to facilitate the transfer of adjuvant arthritis [4], experimental autoimmune thyroiditis [5], and antilisterial immunity [6].

Application of the principle of in vitro conditioning with antigen to the adoptive transfer of DTH should markedly diminish the number of cells necessary for a positive transfer. With this approach, it should be possible to define the cells involved in the adoptive transfer phenomenon by manipulations during the in vitro step. The objectives of the first phase of this work were 1) to define the conditions for adoptive transfer of DTH in rats using in vitro conditioned cells and 2) to study the genetic restriction of the phenomenon. Toward that goal, we report here that as few as 2×10^7 in vitro conditioned spleen cells transfer DTH in a genetically restricted fashion.

Recently, we proposed a hypotheses in which vascular endothelial cells (VE), rather than or in addition to bone-marrow-derived cells, play an integral part in antigen presentation in cell-mediated immune phenomena, including DTH [7]. The hypothesis suggests that VE present antigen in the context of the Ia on the lumenal surface of capillary vessels. Antigen-specific T cells trigger the antigen-armed VE, resulting in the release of factors that lead to the subsequent sequelae known collectively as DTH. The reason for proposing the hypothesis was that we were unable to envisage the events that would lead to the recruitment of a large number of mononuclear immunocompetent cells in the vascular compartment and to their subsequent transvascular migration to the antigen site, without taking into account the role of the endothelial barrier between these compartments. Since the frequency of antigen-specific T cells in the immune individual is low (10^{-3}–10^{-5}), thousands of cells (a number approximating the reciprocal of that frequency, 10^3–10^5) would have to arrive at the site of antigen before a single antigen-specific T cell would be present. Recent studies [8–11] have shown that vascular endothelial cells interact with lymphocytes and are able to present antigen to sensitized T cells under genetically restricted conditions in vitro, and therefore behave as antigen-presenting cells (APC) in a manner similar to bone-marrow-derived APC (macrophages, dendritic cells, etc). In order to test the validity of this hypothesis in vivo, we used the adoptive transfer system, where DTH was passively transferred to chimeric rats that were constructed so that bone-marrow-derived cells and non-bone-marrow-derived cells were of different RT-1 haplotypes (RT-1 is the MHC of the rat). The results indicate that when the antigenically naive recipients received immune donor lymphocytes, DTH responses were observed only when donor cells and recipient non-bone-marrow-derived cells shared a RT-1 haplotype. Transfer of DTH was not observed even when donor cells

and recipient bone-marrow-derived cells were compatible if the non-bone-marrow-derived cells (endothelial cells) were histoincompatible.

MATERIALS AND METHODS

Animals

Female rats at least 10 wk old were used. Lewis/SCN rats were obtained from Charles River Breeding Laboratories (Wilmington, MD) and Brown Norway, ACI, and Buffalo rats from Harlan-Sprague (Walkerville, MD).

Immunization

One milligram of keyhole limpet hemocyanin (KLH) or 50 μg of guinea pig myelin basic protein (MBP) was emulsified in complete Freunds' adjuvant (CFA) containing 10 mg/ml of heat-killed *Mycobacterium tuberculosis* H37Ra (Difco Laboratories, Detroit, MI) and injected into the rear footpads.

Ten to 14 days after immunization, animals were ear tested with 100 μg of KLH and 100 μg of PPD in 0.1 ml in opposite ears. At least 5–7 days were allowed for ear swelling to return to normal before any animal was used as a spleen cell donor.

Measurement of DTH

Ear thickness was measured just prior to ear testing and at 24- and 48-hr intervals using a pressure-sensitive micrometer (L.S. Starrett Co., Athol, MA). Ear swelling was defined as the postinjection measurement minus the preinjection measurement. All measurements were obtained in triplicate.

Adoptive Transfer

Rats were sacrificed with ether anesthesia, the spleens were recovered, and a single cell suspension was prepared by mincing the spleen through a 200-mesh wire screen. After being washed twice in RPMI 1640, the cells were place in culture at a density of 10^6 cells/ml in RPMI 1640 with 10% fetal calf serum and 5×10^{-5} M 2-mercaptoethanol. Either PPD or KLH was added to the culture at 1 μg/ml, which was incubated at 37°C in a 5% CO_2 atmosphere for 48 hr.

Following culture, the cells were recovered and washed twice. Viability was determined by exclusion of trypan blue and the cells were reconstituted at 10^7/ml. Cells were transferred by intraperitoneal injection. Three days after adoptive transfer, the recipients' ears were measured and tested with antigen as described above and shown in Figure 1. Following the 48-hr measurement, representative ears were amputated and fixed in formalin for histological examination.

Chimera Construction

Chimeras were constructed by a modification of described methods [12]. Briefly, F_1 bone marrow was aspirated from donor rats and 2×10^7 bone marrow cells injected intravenously (IV) into parental-type recipients. The recipients were pretreated with 1,000 R from a cesium source shortly before bone marrow administration. The irradiated reconstituted recipients were housed in a laminar flow environment for ten days before returning to conventional animal housing. The chimeras were used 4–8 wk after reconstruction (Fig. 2).

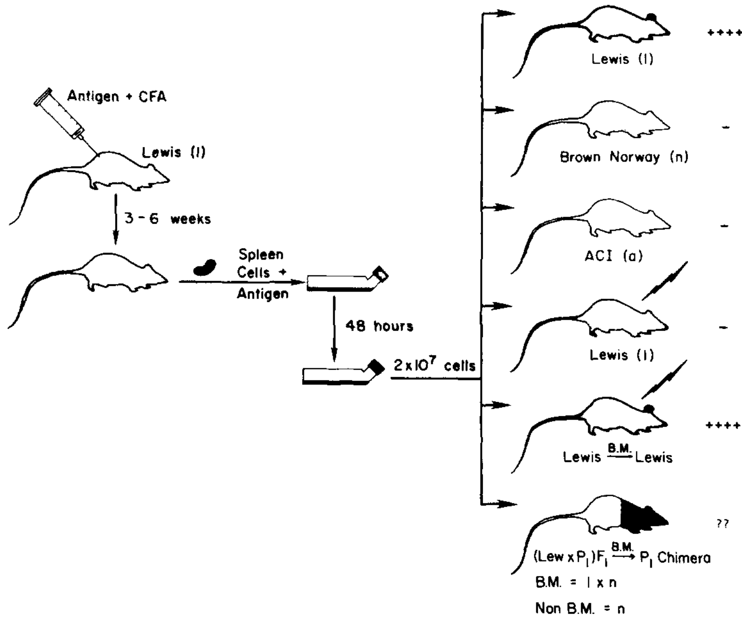


Fig. 1. Steps involved in the adoptive transfer of DTH. The donor animal is first actively immunized and then ear tested. Following a short rest period, spleens are recovered, minced, and a single cell suspension is incubated with antigen for 48 hr. The cells are then washed and injected intraperitoneally. Recipients are tested with 100 μg of the appropriate antigen in one ear and a control antigen in the opposite ear. Measurements of ear swelling are obtained at 24 and 48 hr.

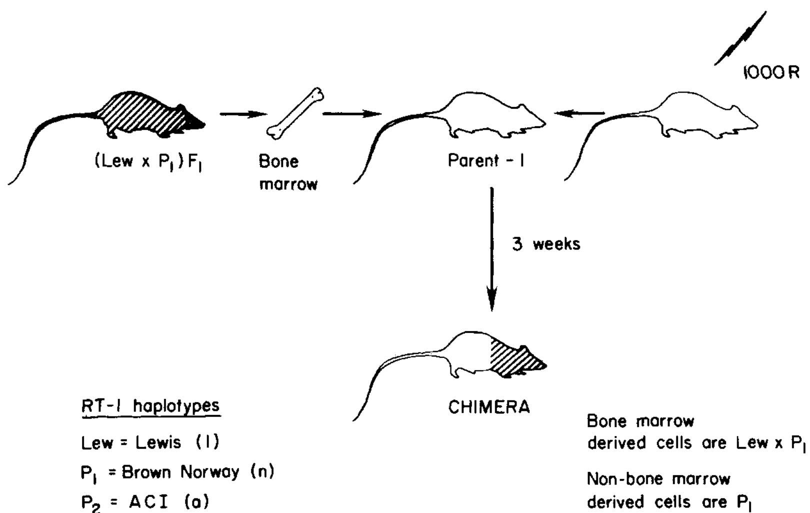


Fig. 2. Steps involved in the construction of chimeras.

RESULTS

DTH in Immunized Rats

Nonimmunized rats were ear tested with diluent (0.9% NACl), KLH, PPD, or MBP to assess background reactivity and the reproducibility of the micrometer measurements. The nonspecific or background swelling is shown in Table 1. Diluent and KLH induced almost no background response. Although both MBP and PPD produced some ear swelling at 24 hr, this reaction diminished by 48 hr. Histologic evaluation of the ears did not reveal any inflammatory changes. The reproducibility of the micrometer measurements was documented by comparison of the mean standard deviation of 25 triplets (1.8 ± 0.8%).

Actively immunized rats developed ear test responses recognizable grossly by erythema and swelling when tested 3–6 wk after immunization. Direct measurement (Table II) of the ears revealed swelling of 0.25–0.30 mm (50–60% increase in thickness), which persisted at 48 hr. Histologic evaluation of amputated ears demonstrated edema and a dense mononuclear cell infiltrate (Fig. 3). Ear swelling after testing with an irrelevant antigen, eg, KLH in CFA/MBP-immunized animals or MBP

TABLE I. Background Responses to Antigens in Nonimmunized Animals (Ear Swelling)*

Rat strain	No. of animals	Antigen (100 μg)	Change in ear thickness (10^{-2}mm + SD) 24 hr	48 hr
Lewis	10	Diluent	2.0 ± 1.2	0.7 ± 1.1
	6	KLH	1.3 ± 1.2	0.7 ± 1.2
	5	PPD	6.0 ± 2.6	−1.0 ± 1.4
	5	MBP	6.2 ± 2.3	0.5 ± 1.0
Brown Norway	2	Diluent	0 ± 0	0.5 ± 0.7
	3	KLH	0 ± 1.2	0.5 ± 1.5
	3	PPD	3.3 ± 1.5	3.3 ± 0.6

*The test antigen was contained in 0.1 ml of diluent and injected intradermally in the ear. Ear swelling was measured using a pressure sensitive micrometer at 24 and 48 hr. Recorded values are the mean of three serially obtained measurements.

TABLE II. DTH Responses to Antigens in Actively Immunized Animals (Ear Swelling)*

Rat strain and Immunogen	No. of animals	Antigen (100 μg)	Change in ear thickness (10^{-2}mm + SD) 24 hr	48 hr
Lewis rats				
KLH/CFA	7	KLH	32.3 ± 5.9	30.8 ± 5.4
	4	PPD	25.5 ± 4.1	25.0 ± 7.6
	3	MBP	5.3 ± 0.6	2.7 ± 0.6
MBP/CFA	4	KLH	2.4 ± 2.1	1.1 ± 0.8
	4	PPD	26.0 ± 2.1	25.5 ± 5.7
	4	MBP	36.8 ± 11	31.7 ± 9.6
Brown Norway				
KLH/CFA	3	KLH	29.0 ± 0	29.5 ± 2.1
	3	PPD	25.5 ± 2.1	30.2 ± 2.8

*The test antigen was contained in 0.1 ml of diluent and injected intradermally in the ear. Ear swelling was measured using a pressure sensitive micrometer at 24 and 48 hr. Recorded values are the mean of three serially obtained measurements.

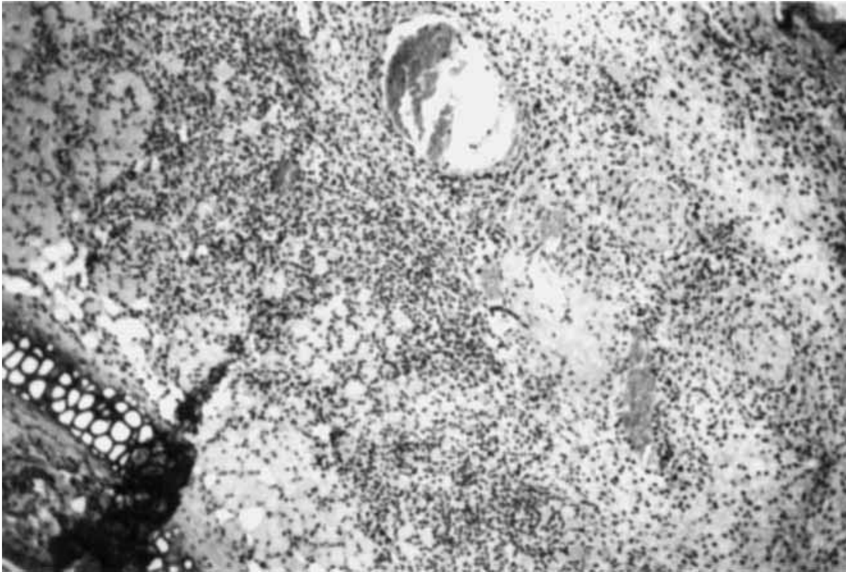


Fig. 3. Sagittal section through the ear of a rat actively immunized with complete Freund's adjuvant and KLH. A dense mononuclear cell infiltrate is present. H & E. × 85.

in CFA/KLH-immunized animals, remained at background levels and histologic evaluation was unremarkable. Histologic examination of ear sections at 6 and 18 hr showed no evidence of Arthus reactions.

Adoptive Transfer of DTH

Adoptive transfer of DTH to naive syngeneic recipients with spleen cells was possible only after in vitro conditioning with the appropriate antigen. The data presented in Table III show that the response is both antigen-specific and directly related to the number of transferred cells. For example, rats receiving 3×10^7 KLH-conditioned cells from a CFA/KLH-immunized donor showed 17.7×10^{-2} mm of ear swelling to KLH (100 μg) at 24 hr but only background levels to PPD (5.7×10^{-2}). At 48 hr, KLH response increased to 20×10^{-2} mm, wherease the response to PPD diminished (4.3×10^{-2}). With optimal transfer conditions, ear swelling after adoptive transfer in Lewis rats is 65–80% of that seen in actively immunized animals. Reliable and easily detectable transfer requires at least 2×10^7 viable spleen cells, although occasional transfers take place with fewer numbers of cells. Based on histologic confirmation (Fig. 4A,B), ear swelling was exclusively due to a mononuclear cell infiltration (lymphocytes and macrophages) and was an accurate method for the detection of DTH (any ear with a response $\geqslant$ 0.10 mm had evidence of cellular infiltration). Equivocal values, 0.06–0.09 mm, may have been secondary to mild DTH, but this could not be confirmed histologically. Significant differences were found between strains of rats used in these experiments. Brown Norway rats did not exhibit a dose-related response for the three cell concentrations tested (Table III). Furthermore, ear swelling after transfer was equal to that seen with actively immunized rats (Table II). The ear reactions in recipients showed no histologic evidence of

TABLE III. Dose Response and Antigen Specificity of DTH Following Syngeneic Adoptive Transfer in Rats with Spleen Cells

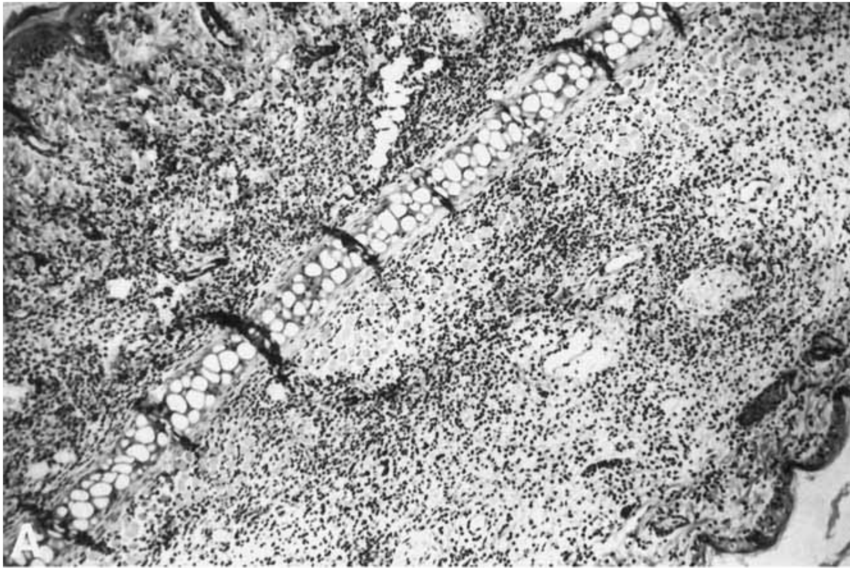
No. of cells transferred[a]	Antigen used in vitro (1 μg/ml)	Change in ear thickness to[b] (10^{-2} mm + SD) KLH (100 μg) 24 hr	KLH (100 μg) 48 hr	PPD (100 μg) 24 hr	PPD (100 μg) 48 hr
Lewis to Lewis					
3×10^7	KLH	17.7 ± 1.2	20.0 ± 1.0	5.7 ± 4.0	4.3 ± 2.1
	PPD	3.3 ± 2.3	2.3 ± 1.2	17.0 ± 2.0	20.7 ± 2.5
2×10^7	KLH	11.3 ± 3.1	9.3 ± 1.2	5.7 ± 0.6	3.3 ± 1.2
	PPD	2.3 ± 1.5	2.7 ± 2.1	14.3 ± 1.5	17.0 ± 4.4
1×10^7	KLH	4.0 ± 1.0	3.3 ± 1.5	1.0 ± 1.0	0 ± 0
	PPD	2.4 ± 1.5	4.0 ± 1.0	4.0 ± 2.0	4.3 ± 1.5
0.3×10^7	KLH	3.6 ± 2.5	5.7 ± 0.6	3.5 ± 0.7	2.3 ± 0.6
	PPD	4.0 ± 1.4	2.0 ± 2.8	5.7 ± 0.6	4.3 ± 1.5
Brown Norway to Brown Norway					
4.5×10^7	KLH	25.7 ± 3.1	23.0 ± 4.6	4.3 ± 3.2	4.0 ± 1.0
3.5×10^7	KLH	26.3 ± 4.2	25.0 ± 4.6	4.7 ± 3.1	4.3 ± 1.5
2.5×10^7	KLH	25.3 ± 10.3	26.3 ± 6.8	7.0 ± 2.6	4.7 ± 1.2

[a]The donors were all immunized with KLH/CFA. The cells were transferred by intraperitoneal administration.
[b]All data points represent three animals.

the participation of Arthus reactivity, and antibodies against the relevant antigen were never detected in recipients.

Additional experiments demonstrate that adoptive transfer of DTH is genetically restricted (Table IV: Lewis vs Brown Norway). Antigen-conditioned spleen cells were equally divided and used in simultaneous syngeneic and allogeneic transfers. Syngeneic recipients displayed unequivocal antigen-specific transfer (20×10^{-2} mm), whereas allogeneic recipients did not demonstrate a response to ear tests (9×10^{-2}). The strain combinations tested were Lewis (haplotype), Brown Norway (haplotype). ACI (haplotype), and Buffalo (haplotype). Only the data from the Lewis-Brown Norway reciprocal experiments is shown. All of the other combinations showed the same consistent pattern. We also tested parental to F_1 combinations with Lewis immune cells. In each case, Lewis immune cells transferred DTH to F_1 combinations that possessed the l haplotype (Table V).

We have shown above that the adoptive transfer of DTH responses is genetically restricted by the RT-1 region of the rat. The following experiments were designed to take advantage of that restriction in the transfer of DTH to PPD and KLH in rats. To clarify the role of bone-marrow-derived cells and non-bone-marrow-derived cells in antigen presentation in the recipients of adoptively transferred cells, bone marrow chimeras were constructed. The rationale was that if chimeric recipients could be constructed so that bone-marrow-derived APC (eg, macrophages, dendritic cells, epidermal Langerhans' cells) differed at the RT-1 region from non-bone marrow derived APC (eg, vascular endothelial cells), immune lymphocytes sharing RT-1 haplotype with either APC type could be used in adoptive transfer to distinguish the relative importance of each APC source in the restriction of the DTH reaction. Chimeras were constructed by lethal irradiation of parental strains (designated P_1, P_2, etc) and reconstitution with F_1 ($P_1 \times P_2$) bone marrow. This procedure produces



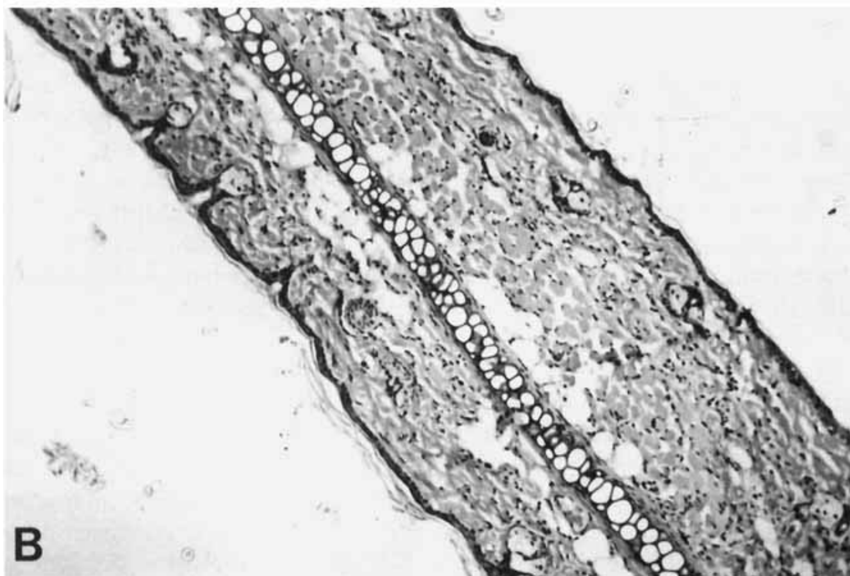


Fig. 4. Adoptive transfer of DTH to the conditioning antigen. Donor spleen cells obtained from an animal actively immunized with CFA/KLH were cultured in vitro with KLH, then transferred by intraperitoneal injection to a previously nonimmunized animal. A) 24 hr after ear test with 100 μg of KLH a dense inflammatory infiltrate is present. B) 24 hr after ear test with 100 μg of PPD no inflammation is seen. Reciprocal reactivity is seen if the conditioning antigen is PPD. H & E. × 85.

TABLE IV. Restriction of DTH Response Following Allogeneic Adoptive Transfer in Rats

Donor and no. of cells transferred[a]	Antigen used in vitro (1 μg/ml)	Change in ear thickness at 24 hr (10^{-2} mm)			
		Lewis recipient (l)		B.N. recipient (n)	
		KLH (100 μg)	PPD (100 μg)	KLH (100 μg)	PPD (100 μg)
Brown Norway donor cells (n)					
4.5×10^7	KLH	1.5 ± 1.0	1.5 ± 1.3	27.6 ± 3.1	2.3 ± 2.1
2.7×10^7	KLH	2.5 ± 1.5	3.3 ± 2.1	28.9 ± 2.8	8.5 ± 1.6
Lewis donor cells (l)					
3.5×10^7	PPD	1.3 ± 1.1	20.0 ± 2.8	3.6 ± 3.1	5.5 ± 1.0
2.0×10^7	KLH	12.8 ± 2.1	3.8 ± 1.2	1.3 ± 1.0	2.0 ± 2.2

[a]Donors all sensitized with KLH/CFA and cells transferred by intraperitoneal administration. Lewis, l haplotype: Brown Norway, n haplotype.

TABLE V. Restriction of DTH Responses Following Adoptive Transfer With Lewis Spleen Cells Conditioned In Vitro With Antigen*

Recipient	RT-1 haplotype	No. animals	Change in ear thickness at 24 hr[a] (10^{-2} mm)
Lewis	l	6	22.2 ± 3.8
Brown Norway	n	6	3.6 ± 2.4
(Lew × BN)F_1	l × n	4	23.1 ± 4.8
(Lew × ACI)F_1	l × a	3	21.0 ± 6.1
(Lew × Buf)F_1	l × b	3	19.2 ± 3.2

*Lew, Lewis; BN, Brown Norway; Buf, Buffalo.
[a]Lewis strain rats were sensitized with KLH/CFA and cells transferred by intraperitoneal administration. Lewis, l haplotype; Brown Norway, n haplotype; ACI, a haplotype.

biologically stable chimeras where the F_1 bone marrow cells reconstitute the irradiated parental rat resulting in a chimeric rat with F_1 ($P_1 \times P_2$) bone-marrow-derived cells and parental non-bone-marrow-derived cells (P_1), including vascular endothelium (Fig. 5). The evidence indicating that the rats were chimeras was twofold: indefinite acceptance of skin grafts from parental haplotype and positive immunofluorescent identification of circulating lymphocytes and macrophages of the F_1 haplotype. The chimeras were then used as recipients of immune cells from each parental haplotype in adoptive transfer experiments. As expected, chimeric recipients of parental immune cells responded in all experiments where immune cells, bone-marrow-derived cells, and endothelial cells shared RT-1 determinants (P_1 to chimera, Table VI). However, when immune cells that were haplocompatible with the chimeric bone-marrow-derived cells but incompatible to the endothelial cells and other non-bone-marrow-derived cells (P_2 to chimera) were transferred, DTH responses were not detected (Table VI).

DISCUSSION

As demonstrated with the transfer of EAE, the key feature of the adoptive transfer of DTH following in vitro conditioning is the dramatic reduction in the

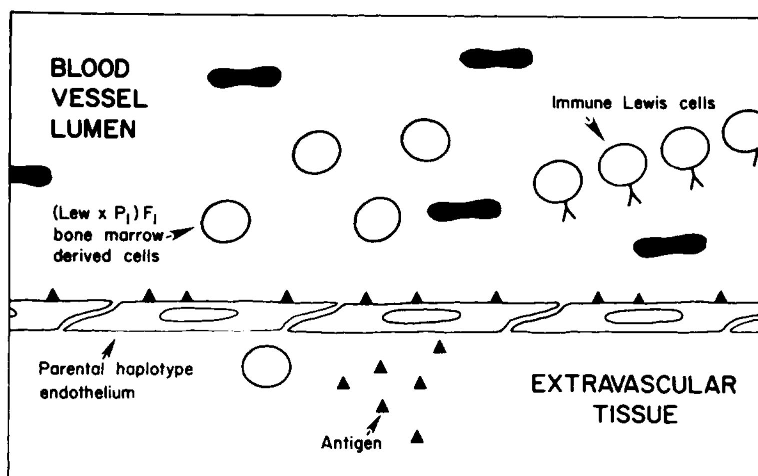


Fig. 5. Graphic representation of the RT-1 haplotype configuration of cells in the chimeric recipients at the time of introduction of immune donor cells.

TABLE VI. Adoptive Transfer of DTH in Chimeric Rats

Chimera construction				Change in ear thickness to PPD (10^{-2} mm + SD)[a]			
				Recipient of P_1 immune cells		Recipient of P_2 immune cells	
Bone marrow donor ($P_1 \times P_2$)	Bone marrow recipient (P_1)	No. of animals	Rest period before use (mo.)	24 hr	48 hr	24 hr	48 hr
(ACI × Le)F_1	ACI	4	1	ND[b]	ND	4.3 ± 6.0	0 ± 4.2
(BN × Le)F_1	BN	4	1	29.0 ± 5.3	31.5 ± 6.4	5.5 ± 6.5	5.8 ± 6.1
(Le × BN)F_1	Le	3	1	18.0 ± 8.5	21.0 ± 13.0	3.0 ± 5.6	1.0 ± 3.0

[a]The left ear tested with 100 μl of PPD (1mg/ml), right ear tested with 100 ul of saline or an irrelevant antigen (KLH, 1 mg/ml). Change in ear thickness is determined by subtracting the change in the right ear from the change in the left ear.
[b]ND, not done.

number of viable cells necessary to effect transfer. Coupled with a reproducible and sensitive method for the measuring of DTH and histologic confirmation of the classic nature of the cellular inflammatory response, adoptive transfer of DTH provides a powerful tool for the investigation of the mechanisms responsible for the development of DTH. We have shown that the addition of an in vitro conditioning step increases the efficacy of transfer of DTH on a cell for cell basis by 25-fold. The transfer is MHC-restricted and requires immunocompetency on the part of the recipient animal [Standage et al, unpublished data]. The mechanism of adoptive transfer can be studied at three levels: (1) requirements of the immune cell donor, (2) analysis of the in vitro step, and (3) requirements of the recipient animal.

Active immunization of the donor animals is possible with a variety of antigens (PPD, KLH, MBP), although CFA was necessary in all cases for positive ear tests with antigen. A positive ear test response (0.20 mm) indentified those animals with active immunity who would be used as donors and serve as a secondary antigenic exposure. The in vitro step involved a 48-hr culture period with the specific antigen.

Ear swelling to test antigens was specific for the conditioning antigen only. The nature of the events that take place in culture are not yet fully understood for either EAE or DTH. Although proliferation appears necessary for the transfer of EAE, it is not known as yet whether this is a requirement for the adoptive transfer of DTH.

It should be possible to investigate which cell subsets are responsible for adoptive transfer through the use of specific monoclonal antibodies. Monoclonal antibodies W3/25 and OX-8 recognize two mutually exclusive subsets of T lymphocytes in the rat. Using affinity-chromatography-enriched populations of thoracic duct lymphocytes, Crum [13] was able to show that cells recognized by OX-8 are incapable of transferring DTH. The W3/25+ T cells were responsible for adoptive transfer of DTH and are therefore analogous to the Lyt 1+2- cells in mice.

Tables IV and V demonstrate genetic restriction for the adoptive transfer of DTH. These data agree with the published reports on the transfer of EAE [14]. Moreover, Wegmann and Hinrichs [15] have shown MBP-sensitive cells from a Lewis donor are capable of transferring clinical EAE to (Lewis $\times$ parental)F_1 recipients, regardless of the haplotype contribution of the other parent.

The chimeric experiments that we have described provide support for the hypothesis that non-bone-marrow-derived cells are an integral part of antigen presentation during the advent of DTH [7]. Bone-marrow-derived APC include macrophages, [16,17], dendritic cells [18,19], and epidermal Langerhans' cells [20,21]. Since the bone-marrow-derived cells were compatible with the immune lymphocytes in the chimeric recipients, the lack of adoptive transfer can only be explained by a sole or an additional requirement of non-bone-marrow derived cells. It is important to point out that radioresistant cells that remain after the chimeric construction do not influence the interpretation of these data. Since an absence of response is used as the end point, residual bone-marrow-derived cells of parental P_1 origin play no role in the response to the P_2 immune cell population and are not a complicating factor in interpreting these data. This also makes the alternative explanation that pre-Langerhans' cells (or other bone-marrow-cells) are "educated" during transit in the skin to express host haplotype unlikely in these experiments. We have not taken into consideration any additional requirements for secondary bone-marrow-derived cells that may require interaction with a histocompatible thymus for proper maturation. The relationships and temporal requirements of only 3 days in vivo tend to make this unattractive, although we can not rule out such an indirect role of cells other than endothelial cells.

One must always be concerned about the possibility of suppression when drawing conclusions from an end point which is the absence of response. In our experiments, suppression of the expected response could be the result of a graft versus host (GVH) reaction in the chimeras from either P_1 or P_2 immune cells. However, GVH was not observed in any of the recipients. Moreover, administration of ten times the cell transfer innoculum was incapable of producing signs of GVH or the accompanying immunosuppression. In additional experiments in which immune cells from both parental haplotype were mixed and simultaneously transferred to chimeric recipients, positive DTH responses were always observed (four of four transfers). Taken together, these observations suggest that suppression does not play a significant role in interpretation of these data.

We have interpreted these experiments to mean that non-bone-marrow-derived cells have a crucial role in the DTH response. Vascular endothelial cells are the most

prominent non-bone-marrow-derived APC candidate due to (1) their large numbers and widespread anatomical distribution, (2) their barrier location between intravascular T cells and the extravascular site of antigen, and (3) their role as APC in vitro [8–10]. Vascular endothelium may be the sole non-bone-marrow-derived APC in DTH responses, but the present experiments do not eliminate the possibility that other non-bone-marrow-derived cells may also act as APC.

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