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The production of contact sensitivity by the injection into the footpads of recipients of the lymph node cells from mice 1 day after painting the skin with contact sensitizing agent: requirement for matching at the major histocompatibility complex between donor and recipient mice

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Summary. Donor mice were painted on the skin of the abdomen with the contact sensitizing agent, oxazolone. One day later $2–5 \times 10^6$ cells from the regional lymph nodes were injected into the footpads of recipient mice. Contact sensitivity was detected 6 days later by challenging the ears of the recipients and measuring the increase of thickness at 24 h. Good contact sensitivity was obtained when CBA cells were injected into CBA mice and BALB/c cells injected into BALB/c mice; the injection of BALB/c (H-2^d) cells into CBA (H-2^k) mice and vice versa failed to give rise to contact sensitivity. Hybrid F_1 cells gave intermediate responses. The contact sensitivity caused by the injection of small numbers of lymph node cells into the footpad is interpreted as a mode of active immunization and the present results show that this only occurs when there is genetic matching at the major histocompatibility complex between the donor and the recipient mouse.

INTRODUCTION

This paper studies the effect of painting the skin of donor mice with contact sensitizing agent and injecting their regional lymph node cells one day later into recipient mice. When the cells are injected into the footpads of the recipient mice, contact sensitivity develops after 3–4 days (Asherson & Mayhew, 1976; Asherson, Zembala & Mayhew, 1977). This is due to an active immunization process and not to the passive transfer of effector cells which mediate contact sensitivity. The evidence for this is that contact sensitivity only appears after a latent period of several days and that the cells still give rise to contact sensitivity after irradiation at 2000 rad. More indirect evidence is provided by the fact that irradiated cells cause DNA synthesis in the regional (popliteal) lymph nodes (unpublished data).

This system of the injection of cells into the footpad might be useful for the analysis of the induction stage of the contact sensitivity reaction. It is first necessary, however, to exclude the possibility that the cells injected into the footpad give rise to contact sensitivity because they carry unaltered contact sensitizing agent on their surface. The fact that dead cells do not cause contact sensitivity when injected into the footpads is some evidence against this view but the interpretation of this observation is difficult as dead cells are handled differently from live cells when they are injected into the recipient. To show that the injected cells played a critical role in the induction of contact sensitivity and

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did not act simply as 'passive transport' for contact sensitizing agent, a study was made of the ability of cells to induce contact sensitivity when the donor and recipient were genetically different. This paper shows that genetic differences between the donor and recipient at the major histocompatibility complex prevented the production of contact sensitivity following injection of cells into the footpad.

MATERIALS AND METHODS

Mice

Specific pathogen-free mice were bred locally at the Clinical Research Centre and used at 8–16 weeks. Mice of one sex were used in any one experiment.

Preparation of donors and injection of cells into the footpad

The skin of the abdomen and lower thorax was clipped with electric clippers and 0·1 ml of 30% ethanolic oxazolone (4-ethoxymethylene-2-phenyloxazolone: BDH, Poole, England) applied. Two drops were also applied to the forepaws. Between 18 and 24 h later the regional (inguinal and shoulder girdle) lymph nodes were taken, dissociated and the cells spun down and washed once. Each recipient mouse was given $2{\cdot}0 \times 10^6$ cells in 0·1 ml divided between both hind footpads.

Assessment of contact sensitivity

Four or six days after the injection of cells into the footpads both sides of both ears of the recipients were painted with 1% oxazolone in olive oil. The increase of thickness of the ear at 24 h was measured with an engineer's micrometer and expressed in units of 10^{-3}cm ± standard deviation. Five mice were used in each group and a group of mice which received no cells was also included as a negative control. Student's two tailed t test was used. Statistically significant implies $P < 0{\cdot}05$.

RESULTS

In the following experiments donor mice were painted on the skin with the contact sensitizing agent, oxazolone, and 1 day later $2{\cdot}0 \times 10^6$ of their regional lymph node cells were injected into the footpads of groups of five recipients. Contact sensitivity was detected in the recipients 6 days later by challenging the ears with 1% oxazolone and measuring the increase in thickness at 24 h in units of 10^{-3} cm.

The first experiment investigated whether the injection of lymph node cells caused contact sensitivity when the donor and recipient mice differed at the major histocompatibility complex (MHC). Table 1 shows that syngeneic injections of CBA (H-2^k) cells into CBA mice and BALB/c (H-2^d) cells into BALB/c mice produced excellent contact sensitivity which was comparable to the reactions seen in mice immunized

Table 1. The production of contact sensitivity by the injection of lymph node cells, taken 1 day after painting with oxazolone, into the footpads of recipients differing at the major histocompatibility locus

Donor of cells*		Recipient of cells	Contact sensitivity in recipient†
CBA	(H-2^k)	CBA (H-2^k)	9·1 ± 1·35
BALB/c	(H-2^d)	CBA	5·8 ± 0·15‡
None	(−ve control)	CBA	5·5 ± 0·38
BALB/c	(H-2^d)	BALB/c (H-2^d)	11·0 ± 3·27
CBA	(H-2^k)	BALB/c	3·3 ± 1·78‡
None	(−ve control)	BALB/c	2·8 ± 1·44

* A total of 2×10^6 cells were injected into the hind footpads of each of five recipients.

† Contact sensitivity was assessed by the increase of ear thickness in units of 10^{-3} cm ± SD on challenge 6 days after the cell injection. The contact sensitivity in CBA and BALB/c mice immunized by painting with 3% oxazolone was 10·2 ± 1·98 and 16·4 ± 4·47 respectively.

‡ These reactions are not significantly different from the negative control.

Table 2. The production of contact sensitivity by the injection of lymph node cells, taken 1 day after painting with oxazolone, into the footpads of recipients sharing the same major histocompatibility locus

Donor of cells	Recipient of cells	Contact sensitivity in recipient
BALB/c	BALB/c	8·5 ± 4·09*
DBA/2	BALB/c	12·4 ± 3·19*
None (−ve control)	BALB/c	0·74 ± 0·25
DBA/2	DBA/2	9·5 ± 1·20*
BALB/c	DBA/2	9·7 ± 1·86*
None (−ve control)	DBA/2	2·6 ± 0·48

* These reactions are not significantly different from each other.

The mice were challanged 4 days after the injection of cells into the footpad.

by painting. However, allogeneic injections from CBA mice into BALB/c recipients and vice versa failed to produce contact sensitivity.

BALB/c and CBA mice differ at both the MHC and other loci. To investigate the importance of the MHC, BALB/c and DBA/2 mice, which have identical MHC but differ at other loci were studied. Table 2 shows that the injection of cells from BALB/c into DBA/2 mice and vice versa gave rise to excellent contact sensitivity. This suggested that the failure of the injection of CBA cells into the footpad of BALB/c mice to cause contact sensitivity was due to differences at the MHC and not at other loci.

To investigate the possibility that BALB/c cells failed to produce contact sensitivity when injected into CBA mice because they were rejected, experiments were undertaken in which F_1 hybrid cells were injected into recipients. Table 3 shows that syngeneic CBA cells gave good contact sensitivity, allogeneic BALB/c cells failed to give rise to contact sensitivity, while F_1 hybrid cells gave intermediate responses. Mixtures of CBA and BALB/c cells behaved like CBA cells alone and there was no evidence that the BALB/c cells interfered with the contact sensitivity produced by CBA cells. Table 3 also shows that parental and F_1 cells gave rise to similar reactions when injected into F_1 hybrids.

In further studies cells from congenic mice were injected into B.10 ($H\text{-}2^b$) mice. Cells from 5R mice, which share the K, IA and IB regions with B.10 mice, gave bigger reactions than those from 4R mice which share the IB, IC, S and D regions (Table 4). Similar results were obtained in a second experiment.

Table 3. The production of contact sensitivity by the injection of lymph node cells taken one day after painting with oxazolone, into the footpads of recipients: studies with F_1 hybrid cells

Donor of cells		Recipient of cells	Contact sensitivity in recipient Exp. 1	Exp. 2	Exp. 3†
CBA	($H\text{-}2^k$)	CBA ($H\text{-}2^k$)	8·6 ± 2·49	8·9 ± 1·63	6·5 ± 0·72
BALB/c	($H\text{-}2^d$)	CBA	3·9 ± 1·25	3·8 ± 0·77	
(CBA × BALB/c) F_1		CBA	5·8 ± 1·62	6·1 ± 1·39	4·9 ± 1·55
*CBA + BALB/c		CBA		10·4 ± 2·69	
None	(−ve control)	CBA	3·9 ± 1·19	4·2 ± 0·97	2·1 ± 0·80
CBA	($H\text{-}2^k$)	(CBA × BALB/c) F_1	7·6 ± 1·94		
BALB/c	($H\text{-}2^d$)	(CBA × BALB/c) F_1	7·8 ± 0·76		
(CBA × BALB/c) F_1		(CBA × BALB/c) F_1	7·2 ± 1·67		
None	(−ve control)	(CBA × BALB/c) F_1	3·6 ± 1·40		

* 2×10^6 CBA and 2×10^6 BALB/c cells injected
† Mice challenged 4 days after the injection of cells into the footpads in Exp. 3.

Table 4. Production of contact sensitivity by the injection of lymph node cells taken one day after painting with oxazolone, into the footpads of congenic B10 recipients

Donor of cells*	K	IA	IB	IJ	IE	IC	S	D	Recipient of cells	Contact sensitivity in recipients
B10	**b**	**b**	**b**	**b**	**b**	**b**	**b**	**b**	B10	8·0 ± 1·10
B10A (4R)	k	k	**b**	**b**	**b**	**b**	**b**	**b**	B10	5·8 ± 0·41
B10A (5R)	**b**	**b**	**b**	k	k	d	d	d	B10	8·5 ± 0·81
None (−ve control)									B10	2·3 ± 0·74

The regions of the MHC shared between the donor and recipient are in bold. The reactions produced by 4R cells are significantly smaller than those produced by 5R cells.
* 5×10^6 cells were injected into the footpads.

DISCUSSION

This paper studies the occurrence of contact sensitivity when cells from the regional lymph nodes of mice are taken 1 day after painting with the contact sensitizing agent oxazolone, and injected into the footpads of recipient mice. The occurrence of contact sensitivity is subject to genetic restrictions. The recipient mice develop little or no contact sensitivity if the major histocompatibility complex of the donor and recipient is different. In particular CBA ($H\text{-}2^k$) and BALB/c ($H\text{-}2^d$) mice gave no reactions when lymph node cells from one strain were injected into the other although syngeneic injections gave good reactions. Non-MHC differences appeared unimportant and good reactions are found when cells are injected from BALB/c into DBA/2 mice and vice versa. These strains have the same MHC ($H\text{-}2^d$) but differ at other loci. More detailed analysis in congenic mice suggested that the left hand side of the MHC was more important than the right hand side.

These findings are consistent with other observations on the requirement for genetic matching. There is a requirement of matching in the K or D regions between the sensitizing and target cells in the killing of virus infected and hapten modified cells (Doherty, Gotze, Trinchieri & Zinkernagel, 1976). Matching in the I-A region is required for the collaboration of macrophages and T cells, and T and B cells in the primary antibody response (Erb & Feldmann, 1976), for the intracellular killing of bacteria (Zinkernagel, Althage, Adler, Blanden, Davidson, Kees, Dunlop & Schreffler, 1977) and for the passive transfer of delayed hypersensitivity to fowl gammaglobulin. Matching in the K, D or I regions is required for the passive transfer of contact sensitivity (Vadas, Miller, Whitelaw & Gamble, 1977).

There are several possible explanations for the genetic restriction found in the present system. It is unlikely that the restriction is due to the rejection of allogeneic cells when they are injected into the footpad. This is because F_1 cells give rise to contact sensitivity while allogeneic cells fail to do so, although both are liable to be rejected.

It is probable that the immediate stimulus for the induction of contact sensitivity is hapten associated with syngeneic major histocompatibility complex antigens. The need for syngeneic MHC antigen may arise for several reasons.

(a) The mouse may have a repertoire of T cells which recognize oxazolone linked to syngeneic MHC antigens but fewer or no cells which recognize oxazolone linked to allogeneic MHC antigen. (See Zinkernagel, Callahan, Althage, Cooper, Klein & Klein, 1978).

(b) Contact sensitivity may show carrier specific for the MHC antigens, i.e. when contact sensitivity is induced by oxazolone associated with a particular MHC antigen the skin reaction can only be elicited by oxazolone associated with the same antigen (Miller, Vadas, Whitelaw & Gamble, 1976).

(c) The cell collaboration between the injected and recipient cells needed for the induction of contact sensitivity may only occur when these cells show matching of the MHC.

The findings summarized in the introduction show that the contact sensitivity which occurs after the injection of cells into the footpad is due to cells which actively immunize the recipient and not to the transfer of effector cells. The view might be held that this contact sensitivity was simply due to the injection of unaltered contact sensitizing agent which was trivially injected together with the cells, and that the properties of the injected cells were of little importance. This view is excluded, however, by the finding of genetic restriction.

At a practical level the injection into the footpad of T cells exposed to transplantation antigen *in vitro* has been used to analyse the induction of graft rejection and cytotoxic T cell responses (Cohen & Livnat, 1976). The present conclusion that the immunization process initiated by the injection of cells into the footpad is not due to the trivial carry-over of unaltered contact sensitizing agent should facilitate a similar analysis of the induction of contact sensitivity.

REFERENCES

ASHERSON G.L. & MAYHEW B. (1976) Induction of cell-mediated immunity in the mouse: circumstantial evidence for highly immunogenic antigen in the regional lymph nodes following skin painting with contact sensitizing agents. *Israel J. Med. Sci.* **12,** 454.

ASHERSON G.L., ZEMBALA M. & MAYHEW B. (1977) Analysis of the induction phase of contact sensitivity by footpad transfer of regional lymph node cells. Macrophages and radioresistant T lymphocytes induce immunity. *Immunology,* **32,** 81.

COHEN I.R. & LIVNAT S. (1976) The cell-mediated immune response: interactions of initiator and recruited T lymphocytes. *Transplant Rev.* **29,** 24.

DOHERTY P.C., GOTZE D., TRINCHIERI, G. & ZINKERNAGEL

R.M. (1976) Hypothesis: models for recognition of virally modified cells by immune thymus-derived lymphocytes. *Immunogenetics*, **3**, 517.

ERB P. & FELDMANN M. (1976) The role of macrophages in the generation of T-helper cells. II. The genetic control of the macrophage–T-cell interaction for helper cell induction with soluble antigens. *J. exp. Med.* **142**, 460.

MILLER J.F., VADAS M.S., WHITELAW A. & GAMBLE J. (1976) Role of major histocompatibility gene products in delayed type hypersensitivity. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2486.

VADAS M.A., MILLER J.F.A.P., WHITELAW A.M. & GAMBLE J.R. (1977) Regulation by the H-2 gene complex of delayed type hypersensitivity. *Immunogenetics*, **4**, 137.

ZINKERNAGEL R.M., ALTHAGE A., ADLER B., BLANDEN R.V., DAVIDSON W.F., KEES U., DUNLOP M.B.C. & SHREFFLER D.C. (1977) H-2 restriction of cell mediated immunity to an intracellular bacterium. Effector T cells are specific for *Listeria* in association with H-2I region coded self-markers. *J. exp. Med.* **145**, 1353.

ZINKERNAGEL R.M., CALLAHAN G.N., ALTHAGE A., COOPER S., KLEIN P.A. & KLEIN J. (1978) On the thymus in the differentiation of 'H-2 self-recognition' by T cells: evidence for dual recognition. *J. exp. Med.*, **147**, 882.