**Brief Definitive Report**

# COOPERATION ACROSS THE HISTOCOMPATIBILITY BARRIER: *H-2*$^d$ T CELLS PRIMED TO ANTIGEN IN AN *H-2*$^d$ ENVIRONMENT CAN COOPERATE WITH *H-2*$^k$ B CELLS*

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The failure of histoincompatible T and B lymphocytes to cooperate in antibody formation has been reported by a number of workers (1–3). This has lead to the concept that T and B cells must express the products of identical "cell interaction" genes for cooperation to occur (4). It is suggested that such genes lie in the *I* region of the major histocompatibility complex (MHC). This region is involved in many other immunological phenomenon and in experiments studying T- and B-cell cooperation it is important to account for the antigens coded for by the *I* region, which provoke a strong alloaggressive reaction. Experiments in which such reactions have been eliminated by one way or another, have shown that successful cooperation can readily occur between histoincompatible cells (5–8). From these experiments one can conclude that there is no obligatory requirement for identity at the MHC for T-B cell cooperation to occur. In certain of these experiments T cells, tolerant of the MHC antigens carried on B cells of another strain, were primed to antigen in an environment containing cells carrying the same MHC antigens as the B cells with which they subsequently successfully cooperated. Thus *H-2*$^d$ T cells if primed in a chimeric animal in the presence of *H-2*$^k$ T cells will cooperate equally well with *H-2*$^d$ or *H-2*$^k$ B cells. Consequently it is possible that T cells will only cooperate with B cells carrying the same MHC antigens as were present when the T cells first encountered antigens. This concept is an extension of the "altered self" hypothesis proposed for cytotoxic T cells but in this case the altered self antigens would be coded for by genes in the *I* region of the MHC (9, 10). In this paper we report experiments in which *H-2*$^d$ T cells from chimeric animals, tolerant of *H-2*$^k$ gene products, were primed with antigen in an essentially *H-2*$^d$ environment. Such helper T cells again cooperated equally well with *H-2*$^d$ or *H-2*$^k$ B cells. If the concept of altered self applies to helper T cells then it is not easily detectable in the interactions of T cells with their target B cells.

## Materials and Methods

*Animals.* Mice of strains AKR, CBA/Ca, BALB/c, C3H/He, and B10D2 new, were obtained from the Laboratory Centre, Carshalton, Surrey, Great Britain. These strains and F1 hybrids C3H/He × BALB/c were bred in the Department of Pathology, Cambridge University.

*Antisera.* Anti-*H-2*$^k$ serum (B10D2 anti-C3H/He) was raised by skin grafting followed by

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repeated intraperitoneal injections of spleen cells. After the fourth injection mice were bled 7 days after each subsequent injection and the sera pooled. Anti-Thy 1.2 serum (AKR anti-CBA) was raised by repeated weekly injections of $10^8$ CBA thymocytes. After the fourth injection mice were bled 7 days after each subsequent injection and the sera pooled. Antisera were used as follows. Anti-Thy 1.2 serum diluted to 1:10 was mixed with $10^8$ spleen cells and left on ice for 30 min. The cells were washed and treated at 37°C for 30 min with 2 ml of a 1:6 dilution of guinea pig complement (C) previously absorbed with BALB/c and CBA spleen cells (4 spleens/1 ml of C). The anti-*H-2*$^k$ serum was used similarly except that 3 ml of a 1:10 dilution was used for each aliquot of $10^8$ spleen cells.

*Source of Hapten-Primed B lymphocytes.* Spleen cells from CBA/Ca mice primed about 3 mo previously with 500 $\mu$g trinitrophenyl hapten (TNP)-ovalbumin in Freund's complete adjuvant (FCA) and then boosted with 100 $\mu$g of antigen before use or from BALB/c mice primed and boosted similarly with TNP-keyhole limpet hemocyanin (KLH) were treated with anti-Thy 1.2 serum and guinea pig C. The cells were then washed and spun through fetal calf serum to remove the majority of dead cells.

*Chimeric Mice.* Radiation bone marrow chimeras (11) were constructed by reconstituting heavily irradiated (900 rads) $F_1$ hybrids (C3H/He × BALB/c) with bone marrow cells previously treated with anti-Thy 1.2 serum and C. Two types of chimeras were made: (*a*) irradiated $F_1$ mice were reconstituted with $4 \times 10^6$ marrow cells from both parental types [tetra parental bone marrow chimeras (TBMC)] and (*b*) irradiated $F_1$ mice were reconstituted with $4 \times 10^6$ bone marrow cells from only one parental strain BALB/c (semiallogeneic chimeras). In both cases the reconstituted mice were left for 3 mo before being used. The *H-2*$^d$ spleen cells from the semiallogeneic chimeras were shown to be unreactive to *H-2*$^k$ cells by the following criteria. Firstly in the presence of *H-2*$^k$ B cells they failed to generate an allogeneic helper effect and secondly they failed to inhibit *H-2*$^k$ spleen cells responding to a thymus-dependent antigen. In each assay up to at least a 20-fold excess of semiallogeneic cells was used over the number of normal *H-2*$^d$ cells which showed a detectable effect.

*Source of KLH-Primed Helper T Lymphocytes.* Helper T cells were obtained from the spleens of conventional CBA and BALB/c mice which had been primed 7 days previously with 500 $\mu$g of KLH in FCA. The T cells were purified by passage through nylon wool columns and erythrocytes removed by treatment with ammonium chloride. Helper T cells were also obtained from chimeric mice. In this case, the erythrocytes and dead cells were firstly removed by spinning with Ficoll-Hypaque and the floating layer of live cells were treated with anti-*H-2*$^k$ serum and C to kill *H-2*$^k$-bearing cells. The cells were then spun through fetal calf serum and transferred to irradiated BALB/c (*H-2*$^d$) recipients which were subsequently primed with 500 $\mu$g of KLH in FCA. After a further 7 days, T cells were purified from the spleens of these primed mice.

*Assay for Helper T-Cell Activity.* Appropriate numbers of KLH-primed T cells with $1.5 \times 10^7$ ml$^{-1}$ hapten-primed B cells were cultured in 10-$\mu$l vol in wells of Terasaki plates under standard conditions (12) in the presence of 2 $\mu$g/ml of TNP-KLH. 30 identical wells were set up for each combination of cells. After 5 days at 37°C the number of cells making antibody to TNP in each well was determined using conventional plaquing techniques with TNP coupled to donkey erythrocytes as indicator cells. The results are expressed as the number of responding cultures out of 30 and also as the total number of plaques (direct plus indirect) from all 30 wells.

## Results and Discussion

In these experiments the relative capacity of T cells from various sources to act as helper cells was measured using B cells from two different strains of mice [CBA/Ca (*H-2*$^k$) and BALB/c (*H-2*$^d$)]. The helper function of the different T cells was measured in microcultures that assess two different parameters of T-cell function. First the number of positive wells reflects the frequency of helper T cells (13) and second the total number of specific antibody-producing cells gives an indication of the capacity of the T cells to cause B-cell clonal expansion. Table I shows that T cells from conventional mice will only cooperate with syngeneic B cells as has previously been reported. However, *H-2*$^d$ T cells obtained from chimeric animals (TBMC) tolerant of *H-2*$^k$ and primed to antigen in an *H-2*$^d$

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TABLE I

*Comparison of the Ability of Primed T Cells from Different Sources to Cooperate with H-2$^k$ and H-2$^d$ B Cells*

| Source of T cells | No. of positive wells (out of 30) with the following B cells | | Total anti-TNP-PFC in 30 wells with the following B cells | |
|---|---|---|---|---|
| | CBA | BALB/c | CBA | BALB/c |
| BALB/c (*H-2$^d$*), 5 × 10$^4$/well | 2 | 28 | 23 | 2,699 |
| CBA (*H-2$^k$*), 5 × 10$^4$/well | 30 | 0 | 15,806 | 0 |
| *H-2$^d$* cells from a chimeric (TMBC) mouse primed in an *H-2$^d$* (BALB/c) recipient, 5 × 10$^4$/well | 29 | 30 | 2,986 | 3,992 |
| 2.5 × 10$^4$/well | 25 | 25 | 1,402 | 2,471 |
| 1.25 × 10$^4$/well | 16 | 18 | 723 | 774 |
| No T cells | 4 | 2 | 150 | 23 |

T cells were obtained from primed conventional mice or from chimeric (TMBC) mice. In the latter case *H-2$^d$* cells tolerant of *H-2$^k$* gene products were primed to antigen in an *H-2$^d$* recipient. Each recipient received 4.5 × 10$^7$ *H-2$^d$* spleen cells from the chimeric mice. The T cells were prepared and assayed for helper activity as described in the Materials and Methods.

mouse, cooperate equally well, within the limits of culture system, with *H-2$^d$* and *H-2$^k$* B cells. This is true at all concentrations of T cells.

It could be argued that some *H-2$^k$* cells had escaped the antiserum treatment during the purification of *H-2$^d$* cells from the chimeric mice and that these cells were responsible for the response seen with *H-2$^k$* B cells. This seems unlikely as neither *H-2$^k$* nor $F_1$ (*H-2$^k$* × *H-2$^d$*) spleen cells could be primed to generate efficient helper T cells in *H-2$^d$* recipient mice under identical conditions (unpublished data). Alternatively it is possible that contaminating *H-2$^k$* cells may have contributed to the induction of helper T cells in the *H-2$^d$* recipient. In order to reduce the risk of contamination by *H-2$^k$* cells similar experiments were performed using *H-2$^d$* cells obtained from semiallogeneic chimeras (BALB/c irradiated C3H × BALB/c $F_1$). The spleen cells from these mice contained less than 10% *H-2$^k$*-bearing lymphocytes and, as before, were treated with anti-*H-2$^k$* serum and C.

Table II shows that T cells from conventional mice again fail to cooperate across the *H-2* barrier. On the other hand, *H-2$^d$* cells, obtained by anti-*H-2$^k$* treatment of spleens from the semiallogeneic chimeras, after priming in an *H-2$^d$* environment again cooperated equally well with *H-2$^d$* and *H-2$^k$* B cells.

The low numbers of *H-2$^k$*-bearing lymphocytes in these semiallogeneic mice even before treatment with anti-*H-2$^k$* serum, means that the degree of contamination by *H-2$^k$*-bearing cells present during the priming of the *H-2$^d$* cells was indeed extremely low. We consider that this data excludes any requirement for T and B cells to share hypothetical "cell interaction" genes (3, 4, 14). Also, if we assume that the level of contamination of *H-2$^d$* spleen cells by *H-2$^k$* cells was too low to influence the immunization of *H-2$^d$* T cells then the data would not be compatible with an altered self mechanism whereby T cells primed in one *I*-region environment would only cooperate successfully with the target B cells which themselves expressed the same *I*-region gene products.

Rather, if an altered self mechanism is applicable to helper T-cell function then it is more likely to be in the reactions with accessory cells such as the interaction of T cells with macrophage-antigen complexes (15–17). However, our arguments are invalid if the low level of contamination by *H-2$^k$* cells is influential. We are now hoping to minimize contamination even further by double

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TABLE II

*Comparison of the Ability of Primed T Cells from Different Sources to Cooperate with H-2$^k$ and H-2$^d$ B cells*

| Source of T cells | No. of positive wells (out of 30) with the following B cells | | Total no. of anti-TNP-plaque-forming cells with the following B cells | |
|---|---|---|---|---|
| | CBA | BALB/c | CBA | BALB/c |
| CBA (*H-2$^k$*), 1 × 10$^5$/well | 30 | 2 | 5,816 | 17 |
| BALB/c (*H-2$^d$*), 1 × 10$^5$/well | 5 | 29 | 114 | 3,027 |
| *H-2$^d$* cells from a semiallogeneic mouse primed in an *H-2$^d$* (BALB/c) recipient, 2.5 × 10$^4$/well | 29 | 26 | 1,573 | 1,432 |
| No T cells | 9 | 13 | 133 | 643 |

T cells were obtained from primed conventional or from semiallogeneic chimeric mice. In the latter case *H-2$^d$* cells tolerant of *H-2$^k$* gene products were primed to antigen in an *H-2$^d$* recipient. Each recipient received 3 × 10$^7$ *H-2$^d$* cells from the chimeric mice. The T cells were prepared and assayed for helper activity as described in the Materials and Methods.

transfer of *H-2$^d$* chimeric spleen cells into two consecutive sets of *H-2$^d$* recipients.

## Summary

*H-2$^d$* spleen cells derived from either tetraparental or semiallogeneic radiation bone marrow chimeras can be primed to antigen within *H-2$^d$* recipients to generate helper T cells capable of cooperating in a secondary response with equal efficiency with *H-2$^d$* or *H-2$^k$* B cells. Thus it would seem that the cooperative act between T and B cells does not require that the T cell interacts with its target B cells by either cell interaction genes or via an altered self mechanism involving both antigen and the target B-cell *I*-region products. This does not preclude a requirement for associative recognition or altered self in the interaction of helper T cells with accessory cells.

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