

# NATURE OF T-CELL MACROPHAGE INTERACTION IN HELPER-CELL INDUCTION IN VITRO\*

## II. Two Stages of T-Helper-Cell Differentiation Analyzed in Irradiation and Allophenic Chimeras

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Antigens of the major histocompatibility complex (MHC)<sup>1</sup> have been shown to be of major importance in the induction of T-cell responses of various types: T-killer cells recognize H-2K and H-2D antigens, while T cells involved in the delayed hypersensitivity response (DTH), antigen induced proliferation or helper function recognize I region associated (Ia) antigens (1-4). There has been much speculation about the mechanism by which antigen-specific T-cell responses are influenced by MHC structures (5), with models proposed involving a single T-cell receptor structure recognizing a complex of MHC-structure-specific antigen (compound antigenic determinant hypothesis), or dual receptors separately recognizing MHC structures and specific antigen (1, 2, 6-13).

Recently, Zinkernagel and his associates (14, 15) have investigated this question by analyzing the response to viruses of various types of chimeric mice, made by using combinations of parental strain and F<sub>1</sub> mice, and found that the potential reactivity of such cells was determined by the thymus of the host in which the T cells developed, and not by the genotype of the stem cells. The development of a genetically restricted potential reactivity, before exposure to antigen (virus), argued for the existence of distinct receptors for self MHC products (14, 15).

The induction of helper cells in vitro requires interaction with macrophage like accessory cells<sup>2</sup> (16). We found that a macrophage factor, a complex of Ia antigen,

\* Supported by the Swiss Science Foundation, (grant 3 103-0.77), the Imperial Cancer Research Fund, and Medical Research Council.

<sup>1</sup> *Abbreviations used in this paper:* AFC, antibody-forming cells; C', complement; DNP-CGG dinitrophenylated chicken gamma globulin; DNP-SRBC, DNP coated sheep erythrocytes; DTH, delayed type hypersensitivity; GRF, genetically related macrophage factor; H-2, major histocompatibility complex of the mouse, comprising the regions K, I, S, G, D: I region: region of the H-2 complex, subdivided into I-A, I-B, I-J, I-E, I-C subregions: Ia: I region associated antigen: KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; PAA, polyacrylamide; PBS, phosphate-buffered saline; PL, peritoneal exudate; SRBC: sheep erythrocytes; TNP-KLH, trinitrophenylated KLH.

<sup>2</sup> Macrophage-like accessory cells is the term we have used for the antigen presenting cells which interact with T cells in helper cell induction. This term is used as it is clear that populations with this function are nearly all phagocytic, but it is still not known whether all macrophages can fulfill this function at the appropriate differentiation stage. The term macrophage is used as an abbreviation.

and a fragment of antigen, of mol wt  $\approx 55,000$ , which we termed GRF (genetically related factor), adequately replaced the function of the adherent accessory cells (17). GRF only activates T cells which share the I region with the GRF donor strain (18). The mechanism of the genetic restriction of GRF—T-cell interaction (or macrophage—T-cell interaction) has been investigated in a number of ways to exclude the possibility that it was caused by a form of T-cell suppression. No suppressor cells were detected by admixing experiments, use of antisera to kill suppressor cells, or chimeric mice which contain two mutually tolerant lymphoid cell pools (19). T cells from irradiation chimeras made by injecting anti- $\theta$ -treated bone marrow cells of both parental types in lethally irradiated  $F_1$  mice retained their genetic restriction, (self preference) despite the fact that they would have been exposed to both parental MHC antigens in the thymus (19).

These results with chimeric mice appear to be contradictory to those of Zinkernagel et al. (15), which suggest that T cells also learn to recognize as self the other parental MHC antigens present in the thymus. They are also at variance with the results of Miller et al. (20) on DTH. They found that T cells from chimeras injected with antigen lost their genetic restriction. However, the studies of Zinkernagel et al. (15) involve T-killer cells, and those of Miller delayed hypersensitivity T cells, both of which are different from the T cells which interact with GRF or macrophage-like cells in helper cell induction (20, 21). For these various reasons a more detailed analysis of T helper cell induction with T cells from various types of chimeras (e.g.  $P \rightarrow F_1$ ,  $F_1 \rightarrow P$ , allophenic) was performed to analyze the development of the T-cell repertoire. The results indicate that the full development of T-helper cell immunocompetence requires the presence of a radioresistant host cell (presumably thymus epithelium), as previously shown for T killer cells by Zinkernagel. However there was an additional stage for helper cells which depends on macrophage-like cells.

### Materials and Methods

*Animals.* All mice including  $F_1$  hybrids with the exception of the chimeric mice were bred at the Institute for Microbiology, University of Basel.

*Radiation Chimeras.* All the radiation chimeras were prepared at University College, London, using protocols similar to Sprent (22) or Zinkernagel et al. (14, 15). The following combinations were made:  $P \rightarrow F_1$ .  $F_1$  (B10  $\times$  CBA) mice  $\approx 10$  wk old were irradiated with 900 rads using a  $^{60}\text{Co}$  source and intravenously injected with  $10 \times 10^6$  anti-Thy 1 + C'-treated bone marrow cells of C57BL/10 (B10) mice.  $F_1 \rightarrow P$ : B10 mice were irradiated twice 600 rads and 900 rads 2 wk apart, and injected with  $10^7$  anti-Thy 1 + C' treated (B10  $\times$  CBA)  $F_1$  bone marrow cells.  $P_1 + P_2 \rightarrow F_1$ : lethally (900 rads) irradiated  $F_1$  hybrids (either (B10  $\times$  CBA)  $F_1$  or (CBA  $\times$  B10.D2) $F_1$ ) were reconstituted by i.v. injection of  $5 \times 10^6$  anti-Thy1 + C'-treated bone marrow cells of each parental strain. All chimeras were rested for at least 3.5 mo and tested for chimerism before use.

*Allophenic Chimeras.* Allophenic mouse chimeras were produced at the Mammalian Development Unit, University College, London. The references for the methods are detailed elsewhere (23, 24). Briefly, 8-cell stage embryos were taken from the oviducts at the 3rd d of pregnancy (vaginal plug = 1st d). Zonae pellucidae were removed by treatment with 0.5% pronase (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif., in 1% polyvinylpyrrolidone K90 in phosphate-buffered saline [PBS]) and a pair of embryos was aggregated in a culture droplet (11) under paraffin oil with fine forceps. After culturing embryos at 37°C in 5%  $\text{CO}_2$  atmosphere for 24–48 h, mosaic blastocysts were taken out and transferred surgically into a uterus of female mice on the 3rd d of pseudopregnancy.

*Antigens.* The antigens used were keyhole limpet hemocyanin (KLH), donated by Dr. M. Rittenberg, University of Oregon Medical School, trinitrophenylated KLH (TNP-KLH), and

dinitrophenylated chicken gamma globulin (DNP-CGG). Conjugates used had 14 groups of TNP per 100,000 daltons of KLH, and 15 groups of DNP per 150,000 daltons of CGG. DNP-polyacrylamide beads (DNP-PAA), a thymus-independent antigen, was kindly prepared by Dr. M. Baltz, University College, London.

*Immunizations.* Mice were primed intraperitoneally with 100  $\mu$ g DNP-CGG coupled on bentonite (25) and boosted with 100  $\mu$ g soluble DNP-CGG 10 d before use as a source of DNP-primed B cells. For the priming of helper cells mice were given 100  $\mu$ g KLH-bentonite twice at an interval of 2 wk and were then rested for at least 6 wk. Some P  $\rightarrow$  F<sub>1</sub> chimeras received only one i.p. injection of 100  $\mu$ g KLH-bentonite but simultaneously at the same site  $5 \times 10^6$  bone marrow derived macrophages of CBA origin which had been cultured for 7 d in vitro, or  $10 \times 10^6$  anti-Thy1 + C'-treated CBA or B10.D2 peritoneal exudate (PE) macrophages induced with 2% starch 4 d previously. The bone marrow-derived macrophages were kindly provided by Dr. A. Mueller, Ciba-Geigy Ltd. Basel. There was no difference in the outcome of experiments using either bone marrow derived macrophages or PE macrophages.

*Antisera.* (B10.A(5R)  $\times$  LP.RIII)F<sub>1</sub> anti-B10 (anti-H-2<sup>b</sup>, pool D-2), and (B10.A (2R)  $\times$  C3H.SW) anti-C3H (anti-H-2<sup>k</sup>, pool D-32) were kindly provided by the Transplantation Immunology Branch, NIAID, NIH, Bethesda, Md. Antiserum 742G (anti-H-2<sup>d</sup>) was donated by Dr. I. F. C. McKenzie, University of Melbourne, Victoria.

*Treatment with Anti-H-2 Sera.* To test for chimerism  $3 \times 10^5$  spleen or T cells from chimeric or F<sub>1</sub> mice were incubated with 30  $\mu$ l of the appropriate dilution of anti-H-2 sera and 30  $\mu$ l medium for 30 min at 4°C. The cells were then washed and incubated with 60  $\mu$ l rabbit complement (absorbed with mouse spleen and liver cells, diluted 1:2) for 30 min at 37°C, and immediately cooled down in a ice-bath. As control cells were incubated either with complement only or with medium alone. The killing activity of the antiserum was determined by the trypan blue dye exclusion test and calculated as percent of the medium and complement control. All chimeras were tested that way before use in experiments. F<sub>1</sub> cells were used as control to test the activity of the anti-H-2 sera.

To remove one haplotype an appropriate number of chimeric T cells (usually  $\approx 50 \times 10^6$ ) were treated with 50  $\mu$ l of an anti-H-2 serum for 30 min at 4°C, then washed and incubated with 50  $\mu$ l C' for 30 min at 37°C. The cells were then washed twice in excess of medium, counted, and the number of reduction compared to the one obtained by testing for chimerism.

*Cell Preparations.* The preparation and purification of T cells, B cells, and macrophages has been described previously (16, 27).

*Tissue Culture Conditions.* The culture systems as well as the media used have been described in detail elsewhere (16, 19, 26). For helper cell induction or restimulation of in vivo primed helper cells the Mini-Marbrook system (19) was used.  $3 \times 10^6$  nylon wool purified T cells were incubated with KLH and  $5 \times 10^4$  macrophages obtained from the PE. After 4 d a small number of living cells which may include helper cells were added to anti-Thy 1 + C'-treated normal or DNP-primed spleen cells (B cells) and TNP-KLH and incubated for 4 d. For these cooperation cultures either the Mini-Marbrook system was used incubating  $3 \times 10^6$  B cells and  $2 \times 10^5$  helper cells or the Mini-Mishell-Dutton system was utilized setting up  $1 \times 10^6$  B cells and  $1 \times 10^5$  helper cells in Micro Test Tissue culture plates (Falcon 3040, flat bottom wells). Mini-Mishell-Dutton cultures were fed daily with 20  $\mu$ l of a nutritional cocktail consisting of a mixture of 2 vol of medium and 1 vol of fetal calf serum.

*Assaying for Antibody Responses.* The numbers of antibody-forming cells (AFC) against TNP was measured by using DNP-SRBC (TNP and DNP cross-react) coupled with DNP Fab fragments as described previously (27). If unprimed B cells were used only direct (IgM) AFC were determined after 4 d. If primed B cells and secondary helper cells were used IgM as well as IgG AFC were determined on day 4 and day 5. For clarity the IgG AFC assayed on day 5 are usually given in the Tables. DNP-specific AFC were enumerated by subtracting the number of plaques obtained with SRBC from that obtained with DNP-SRBC. The results were given as arithmetic means of the DNP AFC of triplicate cultures  $\pm$  standard error.

*Statistics.* Within each experiment the number of specific AFC/culture were compared to that of the positive control (helper cell control) marked (+) using Student's *t* test. *P* values are marked in the Tables as follows: *P* < 0.005 with \*\*\*, *P* < 0.01 with \*\*, *P* < 0.05 with \*.

*Nomenclature of Chimeric Mice.* F<sub>1</sub>  $\rightarrow$  P, F<sub>1</sub> stem cells into irradiated parental mice. (b  $\times$  k)F<sub>1</sub>  $\rightarrow$  b represents (H-2<sup>b</sup>  $\times$  H-2<sup>k</sup>)F<sub>1</sub> cells injected into an H-2<sup>b</sup> host. P  $\rightarrow$  F<sub>1</sub>, Parental stem cells

TABLE I  
Genetic Restriction of Helper Cell Induction with Unprimed ( $P \rightarrow F_1$ ) Chimeras

Helper cell induction <sup>1</sup>	Cooperation <sup>2</sup>				Anti-DNP-response on day 4 <sup>3</sup> IgM-AFC/ $3 \times 10^6$	
	M $\phi$ ( $5 \times 10^4$ )	HC added	F <sub>1</sub> B cells	Antigen	Exp. I	Exp. II
T cells ( $3 \times 10^6$ ) + KLH (0.1 $\mu$ g)	NIL	+	+	TNP-KLH	103 $\pm$ 49	105 $\pm$ 18
	F <sub>1</sub> (b $\times$ k) (a)	+	+	"	260 $\pm$ 47 <sup>+</sup>	1029 $\pm$ 105
	Chimeric	+	+	"	270 $\pm$ 66	ND <sup>5</sup>
	B10 (b)	+	+	"	260 $\pm$ 43	660 $\pm$ 270
	CBA (c)	+	+	"	130 $\pm$ 40*	180 $\pm$ 120***
		a + c <sup>4</sup>	+	"	247 $\pm$ 69	ND
		b + c <sup>4</sup>	+	"	213 $\pm$ 9	504 $\pm$ 42
NIL	NIL	-	+	"	0	90 $\pm$ 30
NIL	NIL	-	+	NIL	10 $\pm$ 8	165 $\pm$ 105

\* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.005$  for Tables I-VII.

Chimeric mice used were B10  $\rightarrow$  F<sub>1</sub> (B10  $\times$  CBA). Exp. I: unprimed B cells,  $2 \times 10^6$  HC added. Exp. II: DNP-primed B cells,  $10^6$  HC added.

H-2 typing:	Percent killing
Anti-H-2 <sup>b</sup> (D-2)	>95
Anti-H-2 <sup>k</sup> (D-32)	<5
Control	<5

Five experiments of this type have been performed, with concordant results.

<sup>1</sup>  $3 \times 10^6$  nylon wool purified T cells were incubated with KLH (0.1  $\mu$ g/culture) and  $5 \times 10^4$  macrophages for 4 d.

<sup>2</sup> For cooperation  $2 \times 10^6$  or  $10^6$  living cells from the first culture (=HC) were added to either  $3 \times 10^6$  unprimed B cells (with the Mini-Marbrook system) or to  $10^6$  DNP-primed F<sub>1</sub> B cells (with the Mini-Mishell-Dutton system) and incubated with TNP-KLH (0.1  $\mu$ g/culture for unprimed B cells, 0.05  $\mu$ g/culture for primed B cells) for 4 d.

<sup>3</sup> Anti-DNP-response is measured after 4 d and calculated as IgM-AFC per  $3 \times 10^6$  input B cells.

<sup>4</sup> To test for suppressor cells  $2 \times 10^5$  or  $10^5$  cells of culture (a) or (b) were mixed with  $5 \times 10^4$  cells of culture (c) and tested for helper activity in the cooperation culture.

<sup>5</sup> ND, not done.

into an F<sub>1</sub> host eg. b  $\rightarrow$  (b  $\times$  k)F<sub>1</sub> indicated H-2<sup>b</sup> cells injected into an irradiated (H-2<sup>b</sup>  $\times$  H-2<sup>k</sup>)F<sub>1</sub> host. P<sub>1</sub> + P<sub>2</sub>  $\rightarrow$  F<sub>1</sub> indicates stem cells from both parents injected into the F<sub>1</sub> host. a  $\leftrightarrow$  b is an allophenic chimera.

## Results

*P  $\rightarrow$  F<sub>1</sub> Radiation Chimeras.* T cells from unprimed b  $\rightarrow$  (k  $\times$  b)F<sub>1</sub> chimeras were incubated with KLH and macrophages of either F<sub>1</sub>, chimeric or parental mice for 4 d and then tested for helper activity with either normal or DNP-primed F<sub>1</sub> B cells (Table I). The results show that F<sub>1</sub>, chimeric or B10, but not, or only to a very minor degree, CBA macrophages induced KLH-specific helper cells. The failure of CBA macrophages to induce helper cells is surprising in view of the previous observations of Zinkernagel et al. (15) that the donor T cells learn to recognize as self the H-2 haplotype of the host during differentiation. Thus one would have expected helper cell induction with CBA macrophages as well. However their failure to do so was a

TABLE II  
*Helper Cell Induction with Primed (P → F<sub>1</sub>) Chimeras*

Helper cell induction				Cooperation <sup>3</sup>			Anti-DNP-response <sup>4</sup> AFC/10 <sup>6</sup>			
b → (k × b) F <sub>1</sub> T		In vivo priming <sup>1</sup>		In vitro priming <sup>2</sup>		HC added	F <sub>1</sub> B cells	Antigen (μg/culture)	IgM	IgG
KLH		KLH				+	+	TNP-KLH 0.05	75 ± 10	125 ± 55
"		" + F <sub>1</sub> Mφ				+	+	"	485 ± 28	585 ± 105 <sup>+</sup>
"		" + B10 Mφ (a)				+	+	"	640 ± 25	890 ± 150
"		" + CBA Mφ (b)				+	+	"	188 ± **	185 ± 5*
"		" + B10·D2 Mφ (c)				+	+	"	58 ± 18***	65 ± 15**
							(+ B10·D2 Mφ)			
						a + b <sup>5</sup>	+	"	495 ± 30	760 ± 10
						a + c	+	"	450 ± 80	920 ± 20
KLH + CBA Mφ		KLH				+	+	"	75 ± 0	175 ± 5
"		" + F <sub>1</sub> Mφ				+	+	"	298 ± 97 <sup>+</sup>	465 ± 25 <sup>+</sup>
"		" + B10 Mφ (d)				+	+	"	360 ± 25	785 ± 75
"		" + CBA Mφ				+	+	"	295 ± 15	505 ± 15
"		" + B10·D2 Mφ (e)				+	+	"	88 ± 42**	40 ± 10***
							(+ B10·D2 Mφ)			
						d + e	+	"	273 ± 8	575 ± 25
KLH + B10·D2 Mφ		KLH				+	+	"	3 ± 3	3 ± 3
"		" + F <sub>1</sub> Mφ (f)				+	+	"	95 ± 10	113 ± 27
"		" + B10 Mφ (g)				+	+	"	215 ± 50 <sup>+</sup>	283 ± 13 <sup>+</sup>
"		" + CBA Mφ (h)				+	+	"	48 ± 8	68 ± 3***
"		" + B10·D2 Mφ (i)				+	+	"	18 ± 8**	3 ± 3***
							(B10·D2 Mφ)			
						g + h	+	"	158 ± 23	195 ± 10
						f + i	+	"	105 ± 10	133 ± 18
NIL	NIL	NIL	NIL			-	+	"	65 ± 5	15 ± 5
"	"	"	"			-	+	DNP-CGG 0.05	965 ± 115	2,555 ± 45
"	"	"	"			-	+	NIL	53 ± 32	20 ± 0

Mice used were B10 → F<sub>1</sub> (B10 × CBA).

H-2 Typing:	Percent killing
Anti-H-2 <sup>b</sup> (D-2)	96
Anti-H-2 <sup>a</sup> (D-32)	3
Control	<4

This experiment was performed six times with comparable results.

<sup>1</sup> Mice were primed in vivo with KLH (100 μg), KLH, and 5 × 10<sup>6</sup> cultured CBA bone marrow cells as a source of macrophages or KLH and anti-Thy1 and C'-treated B10·D2 PE cells induced with starch 4 d previously.

<sup>2</sup> Nylon wool purified T cells of the primed chimeras were restimulated in vitro with KLH (0.1 μg) and 5 × 10<sup>4</sup> macrophages of the strains as indicated for 4 d.

<sup>3</sup> 10<sup>6</sup> living cells of the first cultures (HC cells) were added to 1 × 10<sup>6</sup> DNP-CGG primed F<sub>1</sub> B cells and incubated with TNP-KLH (0.05 μg/culture) for 5 d.

<sup>4</sup> IgM as well as IgG-AFC were measured and given as numbers of AFC/10<sup>6</sup> input B cells. For detecting IgG-AFC an enhancing serum (rabbit anti-mouse IgG) to develop IgG plaques and an anti-μ serum to depress IgM plaques were added to the assay system.

<sup>5</sup> To test for suppressor cells 10<sup>6</sup> cells of the cultures (a), (d), (f), or (g) were mixed with 5 × 10<sup>4</sup> cells of the cultures (b), (c), (e), (h), or (i) and tested for helper activity in the cooperation culture.

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.005 for all tables.

<sup>+</sup> Positive control.

consistent finding of all experiments of this type performed. CBA macrophages incubated with P → F<sub>1</sub> [b → (k × b)] chimeric T cells did not induce suppressor cells, as shown by admixing experiments (Table I). The CBA macrophages used in these experiments were functional if incubated with CBA T cells and KLH (data not shown).

Similar results were obtained if antigen (KLH) primed P → F<sub>1</sub> chimeric T cells were used in vitro instead of unprimed T cells from such chimeras (Table II). Only F<sub>1</sub> and B10, but not CBA macrophages were effective in restimulation of primed nylon

wool purified T cells with KLH, as tested by their ability to help DNP-primed  $F_1$  B cells to generate IgM as well as IgG antibody-forming cells. The failure of CBA macrophages to restimulate could be due to the fact that in the  $F_1$  hosts  $H-2^b$  stem cells would generate cells which are exclusively of the  $H-2^b$  haplotype, including macrophages. The residual  $(B10 \times CBA)F_1$  macrophages would be very small in number; and thus the T-cell pool would respond to KLH associated with  $H-2^b$  type macrophages, and subsequently then would preferentially respond in vitro to the same antigen-macrophage complex. If this prediction is correct, it should be possible to restimulate with  $H-2^k$  type macrophages as well provided such macrophages are made available during the in vivo priming. This was tested, in  $b \rightarrow (k \times b)F_1$  chimeric mice which were simultaneously injected with KLH (100  $\mu$ g) and purified macrophages of the CBA or B10.D2 strain. As a source of macrophages cultured bone marrow-derived macrophages were used because they are not contaminated with lymphocytes. 6 wk later T cells of these animals were restimulated in vitro with KLH and macrophages of either  $F_1$ , B10, CBA, or B10.D2 mice. Table V shows that under these conditions  $F_1$ , B10, and also CBA, but not B10.D2 macrophages were effective in restimulating  $P \rightarrow F_1$  chimeric T cells. Thus the results indicate, that  $P \rightarrow F_1$  T cells have the potential to recognize both  $H-2^k$  or  $H-2^b$ , but not the unrelated  $H-2^d$  macrophages as self, i.e. they are restricted to cooperation with  $H-2^b$  and  $H-2^k$  type macrophages.

*$F_1 \rightarrow P$  Radiation Chimeras.* Chimeras if properly made, have exclusively donor type lymphocytes once the radiation sensitive cells are replaced, that is they have  $F_1$  T cells, B cells, and macrophages. The difference from a normal  $F_1$  animal is that the  $F_1 \rightarrow P$  T cells have differentiated in this case ( $[b \times k]F_1 \rightarrow b$ ) in the  $H-2^b$  host thymus and thus, according to Zinkernagel's hypothesis should, have learned to recognize the  $H-2^b$  haplotype only as self. To test whether this is indeed the case T cells from unprimed  $(b \times k) F_1 \rightarrow b$  chimeras were incubated with KLH and  $F_1$ , B10, CBA or B10.D2 ( $H-2^d$ ) macrophages for 4 d and then tested for helper activity with unprimed or DNP primed  $F_1$  B cells (Table III). The results show that only  $F_1$  and B10, but not CBA nor B10.D2 macrophages generated helper cells in vitro. There was no evidence for induction of suppressor cells by incubating ( $F_1 \rightarrow P$ ) T cells with KLH and CBA macrophages (Table III), and CBA macrophages were functional since they induced helper cells if incubated with CBA T cells and KLH (data not shown).

Similar results were obtained if T cells from in vivo antigen primed  $F_1 \rightarrow P$  chimeras were tested for restimulation with KLH and  $F_1$ , B10, or CBA macrophages (Table IV). Only  $F_1$  and B10 macrophages were able to restimulate KLH primed T cells. CBA macrophages were not active in restimulation of the same T cells. In that Table the results of the IgG response of  $F_1$ , CBA, and B10 B cells are shown.

The IgM response is not shown for clarity, but was analogous but lower in magnitude than the IgG response. It is evident that  $(b \times k) F_1 \rightarrow b$  T cells primed in vivo cannot be restimulated in vitro with KLH and CBA macrophages as they do not help DNP-primed  $F_1$ , or CBA B cells to mount an IgG or IgM response in the presence of TNP-KLH.

The results indicate that  $F_1$  T cells which differentiate in the  $H-2^b$  host are restricted to cooperation with  $H-2^b$  (or  $F_1$ ) macrophages and will not recognize some of their own alloantigens ( $H-2^k$ ) as self.

TABLE III  
Genetic Restriction of Helper Cell Induction with Unprimed ( $F_1 \rightarrow P$ ) Chimeras

Helper cell induction <sup>1</sup>		Cooperation			Anti-DNP-response on day 4 <sup>3</sup> IgM-AFC/3 × 10 <sup>6</sup>	
T cells (3 × 10 <sup>6</sup> ) + KLH (0.1 μg)	Mφ (5 × 10 <sup>4</sup> )	HC added	F <sub>1</sub> B cells	Antigen	Exp. I	Exp. II
+	NIL	+	+	TNP-KLH	65 ± 35	150 ± 90
+	F <sub>1</sub> (b × k)	+	+	"	310 ± 60*	999 ± 225*
+	B10 (a)	+	+	"	320 ± 50	1,290 ± 60
+	CBA (b)	+	+	"	75 ± 5	270 ± 30**
+	B10.D2	+	+ (B10.D2 Mφ) <sup>6</sup>	"	35 ± 15**	ND
NIL	NIL	a + b <sup>4</sup>	+	"	ND <sup>5</sup>	1,044 ± 75
NIL	NIL	-	+	"	40 ± 0	30 ± 10
NIL	NIL	-	+	NIL	80 ± 60	55 ± 35

This experiment was performed with F<sub>1</sub> (B10 × CBA) → B10 chimeras. Exp. I: normal B cells, 2 × 10<sup>6</sup> HC added. Exp. II: DNP-primed B cells, 10<sup>6</sup> HC added.

H-2 typing:	Percent killing
Anti-H-2 <sup>b</sup> (D-2)	>90
Anti-H-2 <sup>k</sup> (D-32)	>90
Control	<10

This experiment was repeated five times with concordant results.

<sup>1-5</sup> See Table I for \*, \*\*, \*\*\*, and other symbols.

<sup>6</sup> 5 × 10<sup>4</sup> PE macrophages (starch induced) from B10.D2 strain were added into the cooperation culture to provide the same macrophages as for helper cell induction.

TABLE IV  
Genetic Restriction in Helper Cell Inductions with Primed ( $F_1 \rightarrow P$ ) Chimeras

Helper cell induction <sup>1</sup>			Cooperation <sup>2</sup>		Anti-DNP-response of B cells <sup>3</sup> IgG-AFC/10 <sup>6</sup>		
T <sub>KLH</sub> (3 × 10 <sup>6</sup> ) + KLH (0.1 μg)	Mφ (5 × 10 <sup>4</sup> )	HC added	Antigen	Mφ (5 × 10 <sup>4</sup> )	F <sub>1</sub>	CBA	B10
+	NIL	+	TNP-KLH	NIL	200 ± 20	235 ± 25	55 ± 31
+	F <sub>1</sub> (b × k)	+	"	"	975 ± 95*	185 ± 32	335 ± 10
+	B10 (a)	+	"	"	890 ± 190	405 ± 35	290 ± 8
+	"	+	"	B10	ND	800 ± 160 <sup>+</sup>	ND
+	CBA (b)	+	"	NIL	400 ± 30**	165 ± 15**	135 ± 0**
+	"	+	"	CBA	ND	ND	40 ± 20***
NIL	NIL	a + b <sup>4</sup>	"	NIL	1,140 ± 20	ND	ND
+	"	-	"	"	30 ± 10	15 ± 5	5 ± 5
+	"	-	DNP-CGG	"	>6,000	1,675 ± 135	413 ± 15
+	"	-	NIL	"	45 ± 25	65 ± 0	15 ± 7

Chimeric mice used were B10 × CBA F<sub>1</sub> → B10.

H-2 typing:	Percent killing
Anti-H-2 <sup>b</sup> (D-2)	>94
Anti-H-2 <sup>k</sup> (D-32)	>93
Control	<5

This experiment was performed four times with comparable results.

<sup>1</sup> KLH (100 μg) primed, nylon wool purified T cells were restimulated with KLH (0.1 μg) and 5 × 10<sup>4</sup> macrophages for 4 d.

<sup>2</sup> 10<sup>5</sup> HC were added to 10<sup>6</sup> primed (B10 × CBA)F<sub>1</sub>, CBA or B10 B cells and incubated together with TNP-KLH (0.05 μg) for 4 d. As a positive B-cell control, cells were incubated with the primary antigen DNP-CGG (0.05 μg).

<sup>3</sup> Only IgG-AFC per 10<sup>6</sup> input B cells are given in this Table.

<sup>4</sup> See Table II.<sup>4</sup>

TABLE V  
Genetic Restriction of Helper Cell Induction with Unprimed ( $P_1 + P_2 \rightarrow F_1$ ) Chimeras

Helper cell induction <sup>1</sup>			Cooperation <sup>2</sup>			Anti-DNP-response of B cells <sup>3</sup> IgM-AFC/3 × 10 <sup>6</sup>		
T (3 × 10 <sup>6</sup> ) + KLH (0.1 μg)	aH-2 + C'	Mφ (5 × 10 <sup>4</sup> )	HC added	Antigen	Mφ <sup>5</sup> (5 × 10 <sup>4</sup> )	F <sub>1</sub>	CBA	B10·D2
+	aH-2 <sup>d</sup>	NIL	+	TNP-KLH	NIL	107 ± 37	ND <sup>6</sup>	ND
+	"	Chimeric	+	"	"	373 ± 14 <sup>*</sup>	ND	ND
+	"	CBA	+	"	"	497 ± 29	540 ± 174 <sup>*</sup>	67 ± 14
+	"	"	+	"	CBA	ND	ND	220 ± 25 <sup>*</sup>
+	"	B10·D2	+	"	NIL	117 ± 12 <sup>***</sup>	47 ± 14 <sup>**</sup>	40 ± 7 <sup>***</sup>
+	"	"	+	"	F <sub>1</sub> (k × d)	50 ± 23 <sup>***</sup>	13 ± 3 <sup>**</sup>	ND
+	"	"	+	"	B10·D2		7 ± 7 <sup>**</sup>	ND
NIL	NIL	NIL	-	DNP-PAA	NIL	637 ± 93	498 ± 61	327 ± 26
"	"	"	-	NIL	"	30 ± 2	43 ± 3	32 ± 3

This experiment was performed using ((CBA + B10·D2 → F<sub>1</sub> CBA × B10·D2)) mice.

This type of experiment has been performed 12 times with concordant results in four different strain combination of chimeras.

<sup>1</sup> See Table I. Before culturing nylon wool purified T cells were treated with anti-H-2<sup>d</sup> serum and complement to remove the H-2<sup>d</sup> cells.

<sup>2</sup> 2 × 10<sup>6</sup> HC were added to either 3 × 10<sup>6</sup> F<sub>1</sub> (CBA × B10·D2), B10·D2 or CBA B cells of unprimed mice.

<sup>3-5</sup> See Table I.

<sup>6</sup> 5 × 10<sup>4</sup> macrophages of the same strain as used for helper cell induction were added to the other parental haplotype B cells.

<sup>7</sup> 20 μl of a 3% solution of DNP-PAA was added per culture.

The use of F<sub>1</sub>, CBA and B10 B cells and macrophages shows that the B-cell response also requires the presence of the initial priming macrophages, because the response of CBA cells is much reduced, unless B10 macrophages are added to these cultures.

$P_1 + P_2 \rightarrow F_1$  Radiation Chimeras. Unprimed T cells from double chimeras d + k → F<sub>1</sub> can be activated to become helper cells with KLH and macrophages of F<sub>1</sub> or of either of the parental mice. However if T cells of one parental haplotype are removed by, for example, treatment of the T cells with anti H-2<sup>d</sup> and complement, the remaining T cells (which are now of H-2<sup>k</sup> haplotype) are only activated with F<sub>1</sub> and k type but not with d type macrophages and vice versa. This is a consistent finding and independent of the strain combinations used for making chimeras (we have used three combinations, reference 19, and Table V). This observation is very similar to the P → F<sub>1</sub> (b → [kxb]F<sub>1</sub>) experiment in which CBA macrophages are also unable to generate helper cells if incubated with normal P → F<sub>1</sub> T cells and KLH.

However, anti-H-2<sup>k</sup> or anti-H-2<sup>b</sup> and complement-treated T cells from in vivo antigen primed  $P_1 + P_2 \rightarrow F_1$  (CBA × B10) chimeras can be restimulated with CBA or B10 macrophages equally well (Table VI), indicating that there is the potential to respond to the opposite haplotype.

That this result is not due to inadequate anti-H-2 treatment is demonstrated by the fact that a one to one mixture of in vivo primed CBA and B10 T cells treated with anti-H-2<sup>k</sup> and C' only cooperated with DNP-primed F<sub>1</sub> B cells if incubated with KLH and B10 macrophages but not if incubated with CBA macrophages, or vice versa (Table VI). The different behavior of T cells of P → F<sub>1</sub> from  $P_1 + P_2 \rightarrow F_1$  (treated with anti-H-2 + C') chimeras indicates that the donor cells which differentiate into T cells and learn to recognize the H-2 haplotype of the host as self during differentiation in the irradiated host still express preference for their own haplotype, thus being activated more easily by antigen in association with macrophages of their own haplotype than with the other haplotype. This preference may be overcome by in vivo priming, where conditions for activation may be more vigorous than in vitro,



TABLE VI  
Lack of Genetic Restriction with Primed ( $P_1 + P_2 \rightarrow F_1$ ) Chimeras

Helper cell induction <sup>1</sup>		Cooperation				Anti-DNP-response on day 5 <sup>3</sup> (AFC/10 <sup>6</sup> )	
T <sub>KLH</sub> ( $3 \times 10^6$ ) + KLH (0.1 $\mu$ g)	aH-2 + C'	M $\phi$ ( $5 \times 10^4$ )	HC added	F <sub>1</sub> B cells	Antigen	IgM	IgG
k + b—(k $\times$ b)F <sub>1</sub>	aH-2 <sup>b</sup>	NIL	+	+	TNP-KLH	40 $\pm$ 20	100 $\pm$ 0
"	"	F <sub>1</sub> (b $\times$ k)	+	+	"	540 $\pm$ 60*	1,560 $\pm$ 20 <sup>+</sup>
"	"	B10 (a)	+	+	"	670 $\pm$ 130	2,230 $\pm$ 70
"	"	CBA	+	+	"	670 $\pm$ 70	1,920 $\pm$ 60
"	"	B10·D2 (b)	+	+	"	160 $\pm$ 10***	550 $\pm$ 70***
				(+ B10·D2 M $\phi$ ) <sup>5</sup>			
"	aH-2 <sup>k</sup>	F <sub>1</sub> (b $\times$ k)	a + b <sup>4</sup>	+	"	660 $\pm$ 120	2,015 $\pm$ 233
"	"	B10	+	+	"	380 $\pm$ 40	940 $\pm$ 20
"	"	CBA	+	+	"	300 $\pm$ 0	1,180 $\pm$ 15
CBA + B10	aH-2 <sup>b</sup>	NIL	+	+	"	355 $\pm$ 45	905 $\pm$ 165
"	"	B10	+	+	"	5 $\pm$ 5	130 $\pm$ 0
"	"	CBA	+	+	"	20 $\pm$ 10	125 $\pm$ 25
"	aH-2 <sup>k</sup>	NIL	+	+	"	260 $\pm$ 0	825 $\pm$ 175
"	"	B10	+	+	"	10 $\pm$ 0	100 $\pm$ 20
"	"	CBA	+	+	"	155 $\pm$ 15	570 $\pm$ 150
NIL	NIL	NIL	—	+	"	10 $\pm$ 0	125 $\pm$ 25
"	"	"	—	+	DNP-CGG	60 $\pm$ 30	70 $\pm$ 10
"	"	"	—	+	NIL	1,105 $\pm$ 185	2,535 $\pm$ 95
"	"	"	—	+	"	35 $\pm$ 35	30 $\pm$ 15

Mice used were (B10 + CBA  $\rightarrow$  F<sub>1</sub> (B10  $\times$  CBA)).

H-2 typing:	Percent killing
Anti-H-2 <sup>b</sup> (D-2)	34
Anti-H-2 <sup>k</sup> (D-32)	77
Control	<10

This experiment has been performed three times with concordant results.

<sup>1</sup> See Table IV. Nylon wool purified chimeric T cells or a 1:1 mixture of CBA and B10 T cells were treated with either anti-H-2<sup>b</sup> or anti-H-2<sup>k</sup> + C' before setting up into cultures.

<sup>2</sup> See Table IV. 10<sup>6</sup> HC were only transferred to 10<sup>6</sup> DNP-primed F<sub>1</sub> (B10  $\times$  CBA) B cells and TNP-KLH.

<sup>3</sup> IgM as well as IgG-AFC per 10<sup>6</sup> input B cells are given in this Table.

<sup>4</sup> See Table II.<sup>5</sup>

<sup>5</sup> See Table III.<sup>6</sup>

thus expanding a T-cell pool which does not express self preference and may be a minority initially, and thus not detected by in vitro assays.

*Allophenic Chimeras k  $\leftrightarrow$  b.* It was of interest to compare whether T cells of unprimed allophenic mice behaved in a similar fashion to the T cells of irradiation chimeras which are still restricted in their cooperation with self macrophages after anti-H-2 and complement treatment. As the extent of chimerism of allophenic mice can vary (28, and T. Matsunaga, unpublished observation), T cells from an individual mouse as well as cells pooled from two to three allophenic mice were tested. Table VII shows the results from three single allophenic mice. Purified b  $\leftrightarrow$  k T cells treated with anti-H-2<sup>k</sup> and C' can be activated to become helper cells with KLH and allophenic, F<sub>1</sub>, H-2<sup>b</sup> and (in contrast to the irradiation chimeras) also with H-2<sup>k</sup> macrophages. Unrelated macrophages, e.g. H-2<sup>d</sup> type (B10.D2) do not activate b  $\leftrightarrow$  k T cells. The same results were also obtained in the experiments where pooled cells from a few allophenic chimeras were used (data not shown). Thus there is a marked difference in the induction of T helper cells between allophenic and radiation chimeras with respect to the capacity of the helper cells to be activated by macrophages of the other parental haplotype.

TABLE VII  
Lack of Genetic Restriction in Helper Cell Induction with Unprimed Allophenic Chimeras (*k* ↔ *b*)

Helper cell induction		Cooperation				Anti-DNP-response on day 4 <sup>3</sup> IgM-AFC/3 × 10 <sup>6</sup>			
T (3 × 10 <sup>6</sup> ) + KLH (0.1 μg)	aH-2 + C'	Mφ (5 × 10 <sup>4</sup> )	HC added (2 × 10 <sup>5</sup> )	Antigen	Mφ <sup>6</sup> (5 × 10 <sup>4</sup> )	Allophenic			
						15 <sup>7</sup>	16 <sup>7</sup>	20 <sup>7</sup>	CBA/B10 <sup>7</sup>
+	aH-2 <sup>k</sup>	NIL	+	TNP-KLH	NIL	0	47 ± 32	50 ± 12	10 ± 6
+	"	Chimeric	+	"	"	223 ± 44 <sup>4</sup>	350 ± 98	215 ± 15	ND <sup>5</sup>
+	"	B10	+	"	"	ND	ND	160 ± 20	163 ± 57
+	"	CBA	+	"	"	357 ± 15	150 ± 34	143 ± 46	0
+	"	B10·D2	+	"	B10·D2	80 ± 50 <sup>6</sup>	ND	ND	ND
+	aH-2 <sup>b</sup>	NIL	+	"	NIL	13 ± 7	150 ± 58	40 ± 40	20 ± 15
+	"	Chimeric	+	"	"	513 ± 220	463 ± 82	ND	ND
+	"	B10	+	"	"	627 ± 93	360 ± 53	387 ± 59 <sup>4</sup>	17 ± 17
+	"	CBA	+	"	"	ND	273 ± 58	ND	203 ± 47
+	"	B10·D2	+	"	B10·D2	ND	ND	50 ± 10 <sup>6</sup>	ND
NIL	NIL	NIL	-	"	NIL	ND	ND	ND	13 ± 3
NIL	NIL	NIL	-	NIL	NIL	ND	ND	ND	0

Mice used CBA ↔ C57BL/6.

H-2 typing:	Percent killing			
	15	16	20	CBA/B10
Anti-H-2 <sup>b</sup> (D-2)	77	67	48	50
Anti-H-2 <sup>k</sup> (D-32)	52	54	80	47
Control	<30	<32	<27	<5

Four experiments of this type have been performed.

<sup>1</sup> See Table I. Nylon wool purified T cells were treated with either anti-H-2<sup>k</sup> or anti H-2<sup>b</sup> serum and complement. As allophenic chimeras were tested individually and the cell number obtained was not always sufficient, only selected cultures were set up with the T cells obtained after the anti-H-2 treatment.

<sup>2</sup> 2 × 10<sup>6</sup> HC were added to 3 × 10<sup>6</sup> unprimed F<sub>1</sub> (CBA × B10) B cells and TNP-KLH.

<sup>3,5</sup> See Table I.

<sup>6</sup> See Table V.

<sup>7</sup> The code number of the individual allophenic chimeras.

<sup>8</sup> A 1:1 mixture of CBA and B10 T cells were treated with either anti-H-2<sup>k</sup> or anti-H-2<sup>b</sup> serum and complement and incubated for helper cell induction with KLH and either CBA or B10 macrophages. Cell of these cultures were transferred to F<sub>1</sub>B cells like the allophenic T cells.

### Discussion

The use of chimeric mice has facilitated the analysis of T-cell recognition of antigen, and of MHC structures (14, 15, 19, 20, 22, 29-32).<sup>3</sup> Using P ↔ F<sub>1</sub>, F<sub>1</sub> ↔ P, and thymus grafted chimeras, Zinkernagel and his associates (14, 15) proposed that radioresistant thymus epithelial cells were instrumental in determining what MHC structures T cells learned to recognize as self. Thus the usual genetic restriction involved in T-cell killing of virus infected cells was modified in chimeric mice, which learned to recognize the host genotype as self and thus lysed virus infected cells of the host type (15). Having studied a different MHC associated (I region) genetic restriction, in the macrophage T-cell interaction step of T-helper cell induction, and using biparental (P<sub>1</sub> + P<sub>2</sub> ↔ F<sub>1</sub>) chimeric mice as one of the tools to exclude T-cell suppression as the mechanism of the genetic restriction (19) we were interested in analyzing the development of immunological competence and genetic restrictions of helper cells in other types of chimeric mice. The results obtained have led us to propose that there are two stages in the development of functional immune competence in helper cells: the first is a permissive stage which occurs in the thymus and

<sup>3</sup> Kappler, J. W., and P. Marrack. Manuscript submitted for publication.

TABLE VIII  
Summary of Chimeric Experiments

Type of chimeras (T-cell source)	Mφ used for primary induction of T helper cells in vitro				Mφ used for restimulation of KLH-primed T cells in vitro			
	A × B	A	B	C	A × B	A	B	C
A + B → A × BB cells	+++	-	+++	-	+++	+++	+++	-
A cells	+++	+++	-	-	+++	+++	+++	-
Allophenic (A ↔ B)								
B cells	+++	+++	+++	-			Not tested	
A cells	+++	+++	+++	-			Not tested	
A × B → A	+++	+++	+/-	-	+++	+++	+/-	-
A → A × B	+++	+++	+/-	-	+++	+++	+/-	-
A → A × B + B Mφ in vivo			Not tested		+++	+++	+++	-
A → A × B + C Mφ in vivo			Not tested		+++	+++	+/-	-

+++ response very good, statistically highly significant.

+/- response variable from small to none, statistically not significant.

- no response.

A cells—after treatment to kill B cells.

B cells—after treatment to kill A cells.

determines the potential T-cell repertoire. The second, a selective or proliferative stage, determines which of the potential repertoires is expressed at a reasonable (detectable) frequency in the peripheral T-cell pool. This stage depends on macrophage-like antigen presenting cells. The evidence for the two stages in the development of immune competence rests on the results obtained with  $P \rightarrow F_1$  and  $F_1 \rightarrow P$  chimeras as summarized in Table VIII. The latter chimeras have T cells which, despite having H-2<sup>k</sup> alloantigens on their surface, do not interact with H-2<sup>k</sup> (CBA) macrophage-like cells (Table III). This indicates that a radioresistant host cell is critical in determining immunocompetence of T cells, and as T cells develop in the thymus this step presumably occurs there. The defect appears to be absolute, as no help is generated with H-2<sup>k</sup> macrophages in vitro even after in vivo priming of these  $F_1 \rightarrow P$  chimeras, which would contain  $F_1$  macrophage-like cells (Table IV). This is unlike the situation with  $P_1 + P_2 \rightarrow F_1$  chimeras, where self preference is demonstrable in T cells from unprimed mice but not after in vivo priming which expands small clones of cells to detectable levels (Tables V and VI).

Evidence for a second stage in T-cell development comes from the  $P \rightarrow F_1$  chimeras, T cells of which will recognize only antigen associated with the stem cell donor's macrophage-like cells (Table I), unless the chimeric mice are reconstituted with  $F_1$  or the other parental macrophages just before priming in vivo. This maneuver yields a T-cell pool which will respond to antigen and macrophages of the other parental type, demonstrating that T cells capable of recognizing antigen in association with these (H-2<sup>k</sup>) macrophages must have been present, either in small numbers capable of being expanded by priming, or in a partly differentiated state capable of differentiating further in the presence of H-2<sup>k</sup> macrophages. Currently the data available cannot discriminate between these hypotheses, although the former seems to be the most likely. The actual events in the first permissive stage taking place in the thymus are also a matter of speculation (32, 33).

Suppression as a mechanism of nonresponsiveness to a particular type of macrophage is unlikely as mixing nonresponding histocompatible helper cell pools with active helper cells yielded an undiminished response (Tables I-IV, VI).

The major unexpected findings were noted in the biparental  $P_1 + P_2 \rightarrow F_1$  chimeric mice, T cells from such unprimed mice show marked self preference for the cells with which they will cooperate (Table V, reference 19). This result, was unexpected on the basis of the results of Zinkernagel et al. (14, 15, 34) investigating T-cell cytotoxicity or the results of Miller et al., (20) by investigating DTH. However the loss of genetic restriction noted (Table VI) after in vivo priming with antigen is totally analogous with Miller's data and would argue that the self preference is clearly not absolute, and may be a quantitative phenomenon only, with self preference due to a higher frequency of anti-self reactive clones. In tissue culture, the number of T cells used is relatively small, as is the period for T-cell proliferation, thus potentially converting quantitative differences in helper precursor frequencies into apparently qualitative differences.

Since chimeric mice may also be made by the fusion of fertilized eggs at the eight cell stage (23), such allophenic chimeras were thus also used for experiments to investigate the T-cell recognition potential. In contrast to irradiation chimeras, T cells from unprimed allophenic chimeras did not show self preference for the accessory cells with which they will collaborate. There are some differences in the nature of the immune system of these two kinds of chimeras: first, in allophenic mice cellular chimerism is created long before the immune system begins to develop in the embryo, whereas in irradiation chimeras adult bone marrow stem cells are introduced and allowed to differentiate in the irradiated hosts of adult mice. Second, the antigenic composition and density of the microenvironment in which lymphocytes differentiate, such as the thymus epithelium differ. In  $P_1$  (or  $P_1 + P_2$ )  $\rightarrow F_1$  irradiation chimeras, the thymus epithelium expresses both MHC antigens codominantly e.g. ( $H-2^b/H-2^k$ ). On the other hand allophenic thymic epithelium must be a mosaic of patches of the two cellular antigens ( $H-2^b/H-2^b$  and  $H-2^k/H-2^k$ ), and the antigenic density of only one parental haplotype is higher in the latter situation. It can be assumed that the thymus epithelium was chimeric in the allophenic mice, or else they would not have responded to both macrophage-like cell types. Third, irradiation chimeras suffer from the side effects of irradiation, such as the increased susceptibility to infections. It is conceivable that the above may cause the difference between the two kinds of chimeras with respect to the capacity of T-helper cell induction by the macrophages. If we take a view that allophenic chimeras are closer to normal animals than irradiation chimeras in all respects of the properties of the immune system, the apparent lack of self-preference from unprimed allophenic mice makes the possibility of a like-like interaction of MHC structures involved in the T-cell recognition process unlikely.

It should be stressed that in the chimeras used where self-preference was lost this was only to the haplotypes involved in the chimera, never to third party haplotypes (Tables II, III, VI, VIII). This is unlike the results obtained by Pierce et al. (35).

The experiments reported here have resemblances to those recently reported by Sprent (22) who also investigated  $F_1 \rightarrow P$  chimeric T cells. These were primed in vivo, in irradiated  $F_1$  mice, and cooperated in irradiated  $F_1$  mice only with  $F_1$  B cells or B cells H-2 compatible with the strain in which the T cells were raised. These results suggest that there is genetic restriction of T-B cooperation, as it may be expected that the irradiated host's  $F_1$  macrophage-like cells would suffice. However, this need not be the case as the actual antigen presenting cells may be very uncommon macrophages

which may not function well in irradiated mice. In our experiments reported here, *in vitro* induced helper cells were assayed with anti-Thy 1-treated spleen cells of  $F_1$  and both parental strains. In all these experiments, of which only two are shown (Table IV and V), T-B genetic restriction was noted, which was overcome by adding macrophages of the H-2 type involved in the initial priming phase. This is the same result as previously reported using  $F_1$  T cells and P macrophages, both by ourselves (19) or by McDougall and Gordon (36). We cannot easily reconcile the differences between the results obtained with *in vivo* priming and assay of T cells (22, 37) and *in vitro* priming and assay. This is not due to the nature of the response (IgM or IgG) or the degree of B-cell priming, but must be due to the different nature of the helper cells induced or assayed in the two systems. Perhaps the simplest explanation is that the *in vitro* primed helper cells collaborate with B cells by means of antigen-specific helper factors which are not genetically restricted in their effects, (38-40) and are much easier to demonstrate using *in vitro* than *in vivo* assays. In contrast the *in vivo* primed helper cells used by Sprent (22, 37) do not release helper factor, and may collaborate by cell contact. It is striking that a prediction of the model based on Sprent's results, namely that T helper cells recognize the same Ia and antigen on a macrophage surface during helper cell induction, as on the B-cell surface during helper cell expression (T-B cooperation), has not been borne out by the *in vitro* tests of Marrack and Kappler<sup>3</sup> who found that the need for responder B cell (but not responder macrophages) could be circumvented by the use of Con A induced nonspecific helper factors *in vitro*. The latter observations imply that during T-B collaboration *in vitro* T cells do not need to recognize antigen and responder type Ia on the B-cell surface, and would be consistent with a heterogeneity of T-B collaborative mechanisms.

Despite these unresolved complexities, certain conclusions can be reached, namely that the genesis of T-helper cell immunocompetence involves two steps, one dependent on a radioresistant host cell, presumably in the thymus, and the other on macrophage-like cells. The mechanisms of these two stages require further investigation.

### Summary

The genetic restriction in the T-cell-macrophage-like cell interaction in helper cell induction was investigated with allophenic and irradiation chimeras of various types. Using T cells from  $P \rightarrow F_1$  chimeras, there was a restriction of cooperation with the parental haplotype accessory cells, unless the chimeric mice were repopulated with macrophages of the opposite haplotype before priming. T cells from primed or unprimed  $F_1 \rightarrow P$  chimeras only cooperated with recipient type accessory cells. These observations led to the hypothesis that there are two stages in the genesis of immunocompetence of T helper cells, one dependent on the thymus, and the other on peripheral macrophage-like cells. Purified T cells from  $P_1 + P_2 \rightarrow F_1$  irradiation chimeras behaved in an unexpected manner in the unprimed state, preferring to cooperate with their own haplotype macrophages. This self preference was lost after antigen priming *in vivo* and was not noted in allophenic chimeras. This loss of self preference was restricted to the haplotypes represented in the chimeras, and did not extend to third party haplotypes.

While these *in vitro* induced helper cells from chimeric mice show clear genetic restrictions at the T-cell macrophage-like cell interaction, there was no evidence for a matching T-B genetic restriction.

We are grateful to Dr. A. McLaren, Medical Research Council Mammalian Development Unit, for generously offering T. M. working facilities to produce allophenic mice, Miss V. Iliescu for technical assistance, Doctors Rittenberg and Baltz for supplying antigens, Doctors I. F. C. McKenzie and P. Lake for supplying antisera, and our colleagues N. A. Mitchison, M. C. Raff, and E. Simpson for critically reviewing the manuscript.

Received for publication 13 October 1978.

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