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The role of MHC class II antigenic determinants in the function of human antigen binding T8$^+$ cells, monocytes and helper and suppressor factors

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SUMMARY

The role of MHC class II antigens was investigated in the process of antigen binding by T8$^+$ cells and monocytes (Mo) and in the functions of helper factor (HF) and suppressor factor (SF). Monoclonal antibodies (MoAbs) to HLA-DR, DC and SB determinants were used in immunofluorescence, inhibition of antigen binding and affinity chromatography of HF and SF. Indirect immunofluorescence studies suggest that T lymphocytes from peripheral blood of healthy subjects have a small proportion of cells expressing HLA-DR, β chain determinants (1·4–3·8%). These belong predominantly to the T8$^+$ subset of cells (4·6–8·8%), with only a very small proportion in the T4$^+$ cells (0·1–1·8%). However, DC1 on DRw6$^+$ T cells and SB2,3 on any HLA typed cells were found in significantly greater proportion than the DR antigens in both T8$^+$ and T4$^+$ cells, though this was again greater on T8$^+$ (30 and 25%) than T4$^+$ (8·3 and 14·4%) cells. Although Mo had a greatly increased proportion of cells with DR-β chain determinants (27–45%) than the T8$^+$ cells, the converse was found with DC1 and SB2,3 determinants (13·9 and 11·4%). Inhibition of ^{125}I-streptococcal antigen (SA) binding to T8$^+$ cells and to Mo by MoAbs to the class II antigens showed that DR-β chain monomorphic or polymorphic antibodies and DC1 antibodies inhibited binding to both cell types by 66–94%. However, MoAbs to DR-α chains or to the SB2,3 determinant failed to yield significant inhibition. Affinity chromatography studies of HF and SF revealed that the DR-β chain monomorphic and DC1 antibodies bound HF and SF activities and that this was not found with the DR-β chain polymorphic or SB2,3 antibodies. The results of inhibition of ^{125}I-SA binding to T8$^+$ cells and Mo, and absorption of HF and SF by affinity chromatography with MoAbs suggest four categories of recognition of human MHC class II antigenic determinants. (1) Class II determinants shared by the T8$^+$ cells, Mo, HF and SF and recognized by MoAbs to monomorphic β chains (DA6.231) and to DC1. (2) Class II determinants shared only by the SA binding T8$^+$ cells and Mo and recognized by the MoAbs to a polymorphic β chain (DA6.164) and to a monomorphic DR determinant (OK.Ial). (3) Class II determinants shared only by the HF and SF and recognized by the MoAbs to one of the α chains (TAL.1B5). (4) Class II determinants not detected on the two cells or the two T cell factors.

Keywords MHC class II antigens T8$^+$ cells

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INTRODUCTION

Human Ia antigens are expressed on B cells, monocytes, dendritic cells and activated T cells (Winchester & Kunkel, 1979; Evans *et al.*, 1978; Reinherz *et al.*, 1979). The presence of Ia antigen on a small proportion of resting human T cells has been reported by some (Fu *et al.*, 1978; Greaves *et al.*, 1979; Schuurman *et al.*, 1980; Ceuppens, Goodwin & Searles, 1981; Whisler, Wajda & Newhouse, 1983) but not by others (Reinherz *et al.*, 1979; Wilson *et al.*, 1979). However, the evidence is in favour of the presence of Ia antigen on resting T cells because Ia antigen is found with T3, T4 or T8 antigen on double staining and additive assays with monoclonal antibodies (MoAbs) (Ceuppens *et al.*, 1981), some cloned T cells have membrane Ia antigens (Moretta *et al.*, 1981) and T cells can synthesize Ia antigens when activated with mitogens, alloantigens and soluble antigens (Fu *et al.*, 1978; Evans *et al.*, 1978; Reinherz *et al.*, 1979).

The human MHC class II antigens consist of at least three separate, though probably related gene loci: HLA-D or DR (reviewed by Winchester & Kunkel 1979; Bodmer, 1981; Strominger *et al.*, 1981), DC (or MT, MB) (Tosi *et al.*, 1978) and secondary B (SB) cell antigen (Shaw, Johnson & Shearer, 1980). The DR antigens have been characterized into α or heavy chains (34,000) and β or light chains (29,000) (Snary *et al.*, 1977; Springer *et al.*, 1977). The DC1 antigen which reacts with DR1,2,w6 antigens has been similarly characterized (Tosi *et al.*, 1978; Shackleford *et al.*, 1981). The SB antigens are also polymorphic and they are distinguished from DR by identification of recombinant families and MoAbs identify epitopes on some alleles of the SB gene (Shaw *et al.*, 1980, 1982).

The objectives of this work were to use MoAbs to some of the DR, DC and SB antigens, to compare their presence on $T4^+$ HC, $T8^+$ SC, monocytes and helper factor (HF) and suppressor factor (SF). These MoAbs were then used in inhibition studies of antigen binding $T8^+$ cells and monocytes. The MoAbs were also used to characterize SA specific HF and SF by affinity chromatography.

MATERIALS AND METHODS

Cell separation and depletion methods. Specimens of venous blood were withdrawn from 17 healthy subjects whose HLA-DR was determined as described previously (Welsh & Batchelor, 1978). Five subjects were HLA-$DRw6^+$ and 12 were HLA-$DRw6^-$, (nine had DR4 and three had DR 2, 3 or 5). Mononuclear cells were separated by the hypaque-Ficoll method. Adherent cells (Mo) were prepared by adherence of F/T separated cells to plastic plates (Falcon 3002), as described elsewhere (Lehner, 1983a). The adherent cells were removed and characterized. The non-adherent cells were then passed down a nylon wool column to remove B cells (Julius, Simpson & Herzenberg, 1973). T4 depleted cells or T8 depleted cells were prepared by killing T cells with the corresponding MoAb (Ortho Laboratories, Raritan, New Jersey, USA) and rabbit complement (Buxted Rabbit Co. Ltd., Sussex, UK), as described elsewhere (Lehner, 1982).

Phenotypic characterization of cells with antibodies and by phagocytosis. Phenotypic characterization of the separated cell populations was performed by indirect immunofluorescence with three sets of antibodies. (1) Mouse MoAb to human OKT3, -T4, -T5 and -T8 antigens (Ortho Laboratories Raritan, New Jersey, USA). (2) MoAb to HLA-DR (β and α chains), DC1 and SB2, 3 determinants, briefly outlined in Table 1. (3) Goat anti-human F(ab) antiserum (Northest Biomedical Laboratories Ltd.). Aliquots of 10^6 cells were incubated with 5 μl of the antibodies for 30 min at 4°C, washed and then 50 μl of FITC conjugated rabbit anti-mouse IgG (Miles-Yeda Ltd, Rehovot, Israel), or rabbit anti-goat IgG (Nordic Immunological Reagents) was added respectively, and incubated for 30 min at 4°C. After washing the cells were mounted on microscope slides and over 200 cells were counted for membrane fluorescence with a Leitz SM-Lux fluorescence microscope with a Ploem illuminator. Low concentrations of MoAbs were used and any background staining was subtracted by including in all experiments cells stained with FITC conjugated rabbit anti-mouse IgG. Fc receptor binding was further excluded by pre-incubation of

Table 1. MoAbs to HLA-DR, DC and SB antigenic determinants used for characterization of antigen binding $T8^+$ cells and monocytes and helper and suppressor factors

Antibody determinant	Code	Supplied by	Reference
DR monomorphic	OK.Ial	Ortho Laboratories	Reinherz *et al.* (1979)
DR-β chain non-polymorphic	DA6.231	Mr K. Guy	Guy *et al.* (1982), Steel *et al.* (1982)
DR-β chain non-polymorphic	HIG.48	Mr K. Guy	Guy *et al.* (1982), Steel *et al.* (1982)
DR-β chain non-polymorphic	HIG.78	Mr K. Guy	Guy *et al.* (1982), Steel *et al.* (1982)
DR-β chain polymorphic	DA6.164	Mr K. Guy	Guy *et al.* (1982), Steel *et al.* (1982)
DR-α chain	DA6.147	Mr K. Guy	Guy *et al.* (1982), Steel *et al.* (1982)
DR-α chain	TA1.1B5	Dr W.F. Bodmer	Brodsky *et al.* (1980)
DC1 (DRw6,1,2)	Genox 353	Dr W.F. Bodmer Dr. M. Crumpton	Brodsky *et al.* (1980)
SB2,3	ILR1	Dr L. Nadler Dr S. Shaw	Shaw *et al.* (1980)

the T cells and Mo with aggregated human γ-globulin (Cohn fraction II; Sigma, London, UK). There was either no change or a slight increase or decrease in the proportion of cells reacting with any one of the antibodies when pre-incubated with aggregated γ-globulin; the decrease was less than 10% of the total. Mo were assessed for phagocytosis by mixing the cells with latex particles coated with human IgG (Wellcome Reagents) and counting the proportion of cells with more than three ingested particles (Lehner, 1982).

Assessment of antigen binding by autoradiography. SA was prepared from *Streptococcus mutans* (serotype c, Guy's strain) as described previously (Russell *et al.*, 1980). This antigen is predominantly protein in nature, has a molecular weight of 185,000 and two well defined antigenic determinants (SA I/II). The antigen was radiolabelled by the chloramine-T method (Hunter & Greenwood, 1962), using 40 μCi of ^{125}I per 1 μg SA. A modified method of autoradiography was used (Davie & Paul, 1971) as described in detail elsewhere (Lehner, 1983a). Briefly, $T8^+$ cells or Mo were incubated with 1 μg of ^{125}I-labelled SA, either at 4°C (with 0·1% sodium azide) for $T8^+$ cells or at 37°C (without azide) for Mo. Smears were prepared for autoradiography and the slides were examined under the microscope, using a $\times$ 100 oil immersion objective and the total number of silver grains were counted in at least 500 cells, as well as the number of grains in adjacent cell free zones, corresponding to an area of 500 cells. The results were expressed as the net grain count, that is the number of grains counted in 500 cells from which the number of grains in the cell free area was subtracted.

Inhibition of binding of ^{125}I-SA to $T8^+$ cells or Mo by MoAbs to MHC class II antigenic determinants. Inhibition studies were carried out with nine MoAbs by incubation 200 μl of 10^6 cells in TC199 with 10 μl of MoAbs for 30 min at 4°C. The cells were then washed twice in cold PBS, suspended in 100 μl TC199, incubated with ^{125}I-SA and examined for antigen binding by autoradiography, as described above.

In vitro *assay of helper and suppressor activity.* Ficoll triosil separated cells were cultured with 1000 ng SA in HEPES-MEM and 5% FCS in Marbrook flasks for 24 h, as described elsewhere (Lehner, 1982). The cell free supernatant (10^{-2} ml/culture) was assayed in large Costar plates (Cambridge, Massachusetts, USA), with 5×10^6 unprimed CBA mouse spleen cells and 100 ng/ml of DNP-SA I/II for HF activity. In order to assay SF activity, SA-induced mouse HC were added to the co-operative cultures (Lehner, 1982). The anti-DNP antibody forming cells (AFC) were assayed on day 4 in triplicate, using the modified plaque assay with DNP coated SRC (Cunningham & Szenberg 1968). The HF and SF activities were expressed as the number of AFC per culture ($\pm$ s.e.).

Affinity chromatography of HF and SF with MoAbs to the MHC class II antigenic determinants. Each of the nine MoAbs were coupled to cyanogen bromide activated Sepharose 4B (Pharmacia, Uppsala, Sweden) at a concentration of 200–500 μl of the MoAb per ml of beads (Lehner, 1983b). The supernates, containing the putative HF or SF were mixed with the beads; the unbound material

Table 2. Phenotypic characterization of four populations of cells

Cell populations	(n)	Mean ± s.e. per cent of cells T3	(n)	T4	(n)	T8	(n)	F(ab)	(n)	Uptake of latex
T	(10)	77·6±1·1	(16)	47·9±1·3	(16)	37·1±1·6	(11)	0·7±0·4	(14)	0·5±0·2
T4$^+$	(5)	79·8±2·4	(8)	72·3±1·8	(8)	0·6±0·4	(6)	0·02±0·02	(6)	1·3±0·7
T8$^+$	(5)	71·5±2·4	(15)	2·2±0·5	(16)	70·0±1·0	(9)	0·1±0·1	(14)	0·6±0·2
Mo	(5)	2·3±0·4	(13)	0·4±0·1	(17)	2·4±0·5	(16)	1·3±0·3	(18)	71·4±1·8

was recovered by centrifugation and the bound material was eluted from the beads by washing with 3M sodium thiocyanate (British Drug House). The eluted material as well as the unbound material were then assayed for HF and SF activity as described above.

RESULTS

Characterization of cell populations

The T cell population showed 77·6±1·1% T3 and less than 1% of F(ab)$^+$ cells or Mo (Table 2). The T4$^+$ cells consisted of 72·3±1·8% T4$^+$ cells, less than 1% T8$^+$ or F(ab)$^+$ cells and 1·3±0·7% Mo. The T8$^+$ cells consisted of 70·0±1·0% of T8$^+$ cells 2·2±0·5% of T4$^+$ cells and less than 1% of F(ab)$^+$ cells or Mo. The Mo population showed 71·4±1·8% Mo and less than 2·5% of T8$^+$, T4$^+$ and F(ab)$^+$ cells.

Characterization of cells with MoAbs to MHC class II antigenic determinants

In the T cell population 6·0±0·8% reacted with the anti-DR monomorphic MoAb (Table 3), 3·8±1·0% with the β chain monomorphic MoAb (DA6.231) and 3·5±0·8% with the β chain polymorphic MoAb (DA6.164) and less than 2% with the α chain and the two other β chain antibodies. Less than 2% of the T4$^+$ cells reacted with the DR monomorphic and β chain polymorphic MoAb and the proportion of cells reacting with a MoAb to α chains was only slightly higher (2·4%). In contrast the T8$^+$ cell population showed an increase in the proportion of cells reacting with MoAbs to DR monomorphic, β chain monomorphic and α chain determinants than the T cells. A comparison between the T4$^+$ and T8$^+$ cells (Table 3) revealed a significantly greater proportion of T8$^+$ cells showing DR ($t=5{\cdot}295$, $P<0{\cdot}001$) and β chain monomorphic ($t=2{\cdot}689$, $P<0{\cdot}05$) and β chain polymorphic ($t=2{\cdot}495$, $P<0{\cdot}05$) determinants. A further comparison between T8$^+$ cells and Mo revealed a significantly greater proportion of Mo with the DR monomorphic ($t=2{\cdot}163$, $P<0{\cdot}05$) and all four β chain determinants ($P<0{\cdot}01$). In contrast both MoAbs to α chains showed less than 0·5% of cells reacting with Mo and a small increase in the T8$^+$ reacting cells ($P>0{\cdot}05$).

The DC1 MoAb (Genox 353) reacted predominantly with DRw6$^+$ cells (Table 3) and a significantly greater proportion of T8$^+$ cells (30%) than T4$^+$ cells (9·4%; $t=5{\cdot}997$, $P<0{\cdot}001$) or Mo (13·9%; $t=4{\cdot}108$; $P<0{\cdot}01$) expressed DC1 antigen. MoAbs to SB2,3 reacted with 11–14% of T and T4$^+$ cells and monocytes but with 25·1% of T8$^+$ cells; the latter was significantly greater than that of Mo ($t=2{\cdot}659$, $p<0{\cdot}05$). Thus, MoAbs to both DC1 and SB2,3 reacted with a higher proportion of T8$^+$ cells than Mo, in contrast to the proportion of these cells reacting with MoAbs to the DR and β chain determinants.

Inhibition of ^{125}I-Sa binding to T8$^+$ cells and Mo by MoAbs to MHC class II antigenic determinants

Inhibition of binding of ^{125}I-SA to both T8$^+$ cells and Mo was 85 to 99% with the DR and β chain monomorphic (DA6.231) MoAb (Table 4). The two other monomorphic β chains MoAb (HIG.48 and 78) inhibited SA binding to either T8$^+$ cells or Mo by a negligible amount (0·8–8·3%). These results are consistent with the high proportion of T8$^+$ cells reacting with the former but not the

Table 3. The proportion of cells in four populations reacting with MoAbs to DR DC and SB antigenic determinants; the results are given as % mean ±s.e.

Cell populations	DR-β chain					DR-α chain		DC1		SB
	DR monom. (OK.Ial)	monom. (DA6.231)	polym (DA6.164)	monom. (HIG.48)	monom. (HIG.78)	(DA6.147)	(TAL.1B5)	DRw6+ (Genox 353)	DRw6− (Genox 353)	SB2,3 (ILR1)
T	6·0±0·8	3·8±1·0	3·5±0·8	1·4±0·5	1·4±0·5	†0·4±0·2	0·8±0·4	—	—	†10·9±3·5
T4+	*1·0±0·5	*1·8±0·7	*0·1±0·1	—	—	—	2·4±0·8	9·4±1·5	4·4±1·0	14·4±3·7
T8+	14·8±2·4	8·8±1·8	4·6±1·2	5·9±3·8	5·5±4·1	3·9±2·2	2·3±1·3	30·0±3·4	0·8±0·5	25·1±3·6
Mo	26·7±3·2	45·2±4·7	27·2±4·9	29·0±4·3	35·4±5·5	0·4±0·4	0·2±0·2	13·9±2·3	2·9±1·1	11·4±3·5
n	12–17	5–9	5–7	4–6	4–6	5	4–6	4–6	4–6	4–5

* $n=4$; † $n=3$.

Table 4. Inhibition of streptococcal antigen binding to $T8^+$ cells and monocytes by anti-HLA-DR, DC and SB MoAbs

	$T8^+$ cells			Monocytes		
	Net grain count ± s.e.		% Inhibition	Net grain count ± s.e.		% Inhibition
Inhibitor	without Inhibitor	with Inhibitor	± s.e. (*n*)	without Inhibitor	with Inhibitor	± s.e. (*n*)
Ia monomorphic (OK.Ial)	86·6 ± 28·7	2·6 ± 2·6	99·0 ± 1·0 (7)	270·0 ± 87·4	11·4 ± 8·3	95·9 ± 3·7 (7)
β chain (DA6.231)	129·8 ± 47·1	34·5 ± 23·4	84·8 ± 9·5 (4)	343·2 ± 106·6	34·8 ± 32·6	94 ± 43·0(5)
β chain (DA6.164)	129·8 ± 47·1	45·5 ± 25·9	65·8 ± 8·8 (4)	244·8 ± 52·9	32·8 ± 25·1	86·5 ± 8·0 (4)
β chain (HIG.48)	304·5 ± 104·9	324·2 ± 126·0	0·8 ± 0·8 (4)	508·0 ± 101·5	556·0 ± 195·0	8·3 ± 4·4 (3)
β chain (HIG.78)	304·5 ± 104·9	290·0 ± 104·4	5·8 ± 3·1 (4)	508·0 ± 101·5	567·0 ± 172·0	2·3 ± 2·3 (3)
α chain (DA6.147)	163·7 ± 46·2	146·7 ± 25·8	14·7 ± 7·3 (3)	244·8 ± 52·9	225·0 ± 56·6	10·0 ± 7·6 (4)
α chain (TAL.1B5)	270·3 ± 80·8	256·1 ± 83·8	20·4 ± 9·0 (7)	693·0 ± 293·2	672·8 ± 299·4	6·4 ± 2·7 (5)
DC1 (Genox 353)	(a) 46·5 ± 6·9	8·5 ± 7·2	81·8 ± 13·9(4)	120·0 ± 21·7	27·3 ± 12·2	81·0 ± 5·3 (3)
DC1 (Genox 353)	(b)314·4 ± 102·5	337·4 ± 121·6	14·0 ± 8·1 (5)	343·4 ± 89·3	259·4 ± 31·0	18·2 ± 8·1 (5)
SB2, 3 (ILRI)	244·6 ± 109·6	292·2 ± 142·7	12·2 ± 6·1 (5)	523·0 ± 330·0	359·0 ± 165·0	18·0 ± 12·5(3)

Table 5. Immunoadsorption of helper and suppressor factors with MoAbs to DR, DC and SB antigenic determinants; the results are given as mean ± s.e. of antibody forming cells in three to five factors in each group

	Helper factor		Suppressor factor	
Adsorbent	Bound	Not bound	Bound	Not bound
Nil (DNP-SA)	34·1 ± 2·9		29·8 ± 11·7	
Nil (DNP-SA + HF)	183·5 ± 11·0			
Nil (DNP-SA + HC)			255·6 ± 9·7	
Nil (DNP-SA + HC + SF)			44·4 ± 4·8	
Ia monomorphic (OK.Ial)	39·0 ± 5·9	14·0 ± 22·0	198·7 ± 7·8	52·3 ± 12·7
β chain (DA6.231)	173·7 ± 20·3	35·7 ± 11·0	42·0 ± 12·4	189·0 ± 30·3
β chain (DA6.164)	36·7 ± 2·0	186·3 ± 7·8	201·0 ± 6·7	45·3 ± 7·9
β chain (HIG.48)	32·0 ± 9·5	157·7 ± 22·6	177·7 ± 3·9	57·7 ± 9·6
β chain (HIG.78)	59·0 ± 18·0	123·3 ± 8·2	148·7 ± 14·3	54·3 ± 7·2
α chain (DA6.147)	36·7 ± 4·9	159·0 ± 23·1	172·0 ± 4·5	25·3 ± 12·3
α chain (TAL.1B5)	194·3 ± 5·9	30·0 ± 6·8	45·3 ± 6·2	221·3 ± 20·2
DC1 (Genox 353)*	175·7 ± 30·6	45·7 ± 5·7	36·7 ± 7·1	175·3 ± 27·0
DC1 (Genox 353)†	44·7 ± 2·3	197·7 ± 11·8	158·0 ± 21·6	23·3 ± 2·0
SB2, 3 (ILR1)	65·8 ± 17·5	158·2 ± 26·9	136·5 ± 12·9	40·8 ± 6·1

* With DRw6 + cells; † with DRw6 − cells.

latter MoAb (Table 3). However, Mo showed a high proportion of cells binding with the monomorphic β chains MoAb (HIG.48 and 78) and yet SA binding was inhibited by less than 10%. The polymorphic β chain antibody (DA6.164) showed significant inhibition of SA binding to both $T8^+$ cells (65·8%) and Mo (86·5%). However, both α chain MoAbs failed to inhibit SA binding to either cell by more than about 20%.

The MoAb to DC1 also showed a high degree of inhibition of binding to $DRw6^+$, as compared with $DRw6^-$, $T8^+$ cells and Mo (81%; Table 4). However, the single MoAb available to the SB2,3 antigens (ILR1) failed to inhibit SA binding by more than 20%.

Affinity chromatography of HF and SF with MoAb to MHC class II antigenic determinants

Both HF and SF activities were adsorbed by one of the three β chain monomorphic MoAb (DA6.231), by one of the two α chain MoAbs (TAL IB5) and by the DC1 MoAb; the latter with HF or SF from $DRw6^+$ cells only (Table 5). The other three MoAbs to β chains, an α chain and a monomorphic DR MoAb failed to adsorb HF or SF activity. The MoAb to SB2,3 also failed to adsorb any activity (Table 5). The results suggest that selective DR α chain and β chain determinants, as well as DC1 determinants might be present in both HF and SF. However, the SB2,3 determinants, assayed by the available MoAbs have not been detected in either HF or SF.

DISCUSSION

Human peripheral blood T cells, reacted by immunofluorescence with a MoAb to a DR monomorphic determinant (6·0 ± 0·8%) and to two out of four MoAbs to DR-β chains (3·5 and 3·8%; Table 3). The two MoAbs to α chain epitopes reacted with less than 1% of the T cells. These results suggest a progressive decrease in recognition of DR determinants on T cells from DR monomorphic to β chains and α chains epitopes. Although the seven MoAbs to DR are not comparable in concentration or avidity, the differences in the proportion of T cells reacting with them is noteworthy. The results are consistent with finding Ia determinants on resting T cells by others (Yu *et al.*, 1980; Ceuppens *et al.*, 1981; Whisler *et al.*, 1983), although the MoAb used were probably only to some 'framework' or invariant part of DR. A significant difference in reactivity

between the MoAb to the β chain (DA6.231) and the α chain (DA6.147) has been reported previously in PHA and PWM activated T cells (Guy *et al.*, 1982).

Separation of the T cell population into $T4^+$ and $T8^+$ cells revealed a significant increase in the proportion of $T8^+$ cells reacting with MoAbs to DR and β chains but not to an α chain determinant (Table 3). This suggests that the small proportion of DR^+ peripheral blood T cells may belong predominantly to the $T8^+$ subset. This is consistent with the findings that a greater proportion of $T8^+$ than $T4^+$ cells react with a MoAb to an Ia 'framework' determinant (Ceuppens *et al.*, 1981). It should not be surprising to find that a small proportion of 'resting' T cells express DR antigen, as some antigenic stimulation might be inevitable under normal environmental conditions. It should be, however, noted that most of these cells are $T8^+$ cells and whether these cells belong to the suppressor, cytotoxic or contrasuppressor subset is not clear. There is evidence that at least some of these cells might belong to the contrasuppressor subset, as the proportion of $T8^+$ Ia^+ cells is about three times higher in CSC than SC.

It was predictable that a much greater proportion of Mo would react with the MoAb to DR determinants, but there were important differences between them (Table 3). A significantly greater proportion of Mo than $T8^+$ cells reacted with all four MoAbs to the DR-β chains (Mo:T8, 4·9 to 6·4) and to a lesser extent with the DR monomorphic determinant (Mo:T8, 1·8). The converse was found with the two MoAbs to DR-α chains; a lower proportion of Mo than $T8^+$ cells reacted with these MoAbs. Thus, DR determinants might be expressed on the surface of Mo predominantly as β chains and the ratio of β to α chains was up to 226; the corresponding ratio on $T8^+$ cells was only up to 3·8. The paucity of expression of the α chain DA6.147 on the surface of B cells and activated T cells has also been reported by others (Guy *et al.*, 1982). The specificity of the MoAb to DC1 (DR1, 2,w6) was established previously (Brodsky *et al.*, 1980) and confirmed here with $DRw6^+$ cells (Table 3). However, the proportion of $DRw6^+$ cells reacting with the MoAb to DC1 was high for all cells and significantly greater with $T8^+$ cells than with Mo; this relationship is the reverse to that found with the MoAb to DR-β chain determinants. The proportion of cells reacting with the MoAb to SB2,3 (ILR1) was also rather high for all cells, especially $T4^+$ cells (14·4%) but again a significantly greater proportion of $T8^+$ cells than Mo reacted with this MoAb (Table 3). The significance of these quantitative differences between the class II determinants on $T8^+$ cells and Mo is presently not known and presumably they reflect differences in functional activities between the $T8^+$ cells and Mo.

Although these comparative quantitative data have to be regarded with some caution, due to lack of standardization of the concentration and avidity of the MoAb, nevertheless they suggest that some DR monomorphic, DR-β chain, DC1 and SB2,3 epitopes are shared by the class II antigens of $T8^+$ cells and Mo. At least one of the DR-β chain epitopes (DA6.231) is also shared by human peripheral B cells and activated T cells (Guy *et al.*, 1982), so that some DR epitopes are shared by the DR gene products found on B cells, Mo, $T8^+$ cells and activated T cells. Whereas $T4^+$ cells have a negligible proportion of cells with the DR-β chain determinants, there is a significant increase in $T4^+$ cells with DC1 ($P < 0{\cdot}01$) and SB2,3 determinants ($P < 0{\cdot}02$). However, DR-β chain determinants are found in a high proportion of $T8^+$ cells and there is a further significant increase in the proportion of cells with DC1 ($P < 0{\cdot}01$) and SB2,3 ($P < 0{\cdot}01$) determinants. The converse was found with Mo, in that the proportion of cells with DR-β chains was significantly higher than those with DC1 or SB2,3 determinants ($P < 0{\cdot}001$).

The specificity of ^{125}I-SA binding to $T8^+$ cells and to Mo was established previously (Lehner, 1983a). Inhibition of ^{125}I-SA binding by MoAb to HLA-DR, DC and SB determinants showed similar results for $T8^+$ cells and Mo. Three of the seven MoAbs (OK.Ial, DA6.231 and DA6.164) inhibited binding to both cells by 66–99% (Table 4). This applied to two of the four DR-β chain antibodies, but neither of the two DR-α chain antibodies inhibited SA binding by more than 20%. The MoAb to DC1 also inhibited SA binding to $DRw6^+$, $T8^+$ cells and Mo by more than 80%; $DRw6^-$, $T8^+$ cells or Mo were inhibited by DC1 antibodies by less than 20%. Furthermore, MoAbs to SB2,3 showed less than 20% inhibition of $T8^+$ cells or Mo. Clearly, other MoAbs to SB determinants will have to be examined.

It seems therefore that SA binding is related to a number of HLA class II antigens, predominantly DR-β chain and DC1 determinants but not to the DR-α chain or SB2,3 determinant.

Table 6. Four types of MHC class II determinants recognised by antigen binding T8+ cells and monocytes and by affinity chromatography of helper and suppressor factors

		% Inhibition of ^{125}I-SA binding		% Binding	
	MoAbs	T8+ Cells	Monocytes	HF	SF
Type 1	DR-β chain monomorphic (DA6.231)	84·8	94·0	93	95
	DC-1 (Genox 353)	81·8	85·0	95	97
Type 2	DR-β chain polymorphic (DA6.164)	65·8	86·5	2	24
	DR monomorphic (OK.Ia1)	99·0	95·9	3	25
Type 3	DR-α chain (TAL.IB5)	20·4	7·3	100	93
Type 4	SB-2,3 (ILR1)	12·2	18·0	21	53*
	DR-β chain monomorphic (HIG.48)	0·8	8·3	0	34*
	(HIG.78)	5·8	2·3	10	47*
	DR-α chain (DA6.147)	14·7	10·0	2	37*

* Although 34–53% of SF activity bound to these MoAbs, 88–100% of SF activity was found in the corresponding unbound fractions.

Although numerous investigations have been published on the inhibitory effect of antibodies to Ia on presentation of the Ia antigen complex by macrophages to T cells (Unanue, 1981), very few studies have been reported on the significance of Ia in antigen binding to macrophages. However, mouse peritoneal exudate cells binding of intact *Listeria monocytogenes* was not inhibited with antibodies to the I-A haplotype of the mouse used (Unanue, 1981). The present results suggest that binding of SA to both Mo and T8+ cells might be mediated by DR and DC antigenic determinants. Whether this is a direct reaction or one modulating the cell surface antigens needs to be investigated. Affinity chromatography studies of HF and SF revealed that the McAb to a monomorphic DR-β chain (DA6.231) and to DC1 (Genox 353) which inhibited ^{125}I-SA binding to T8+ cells and Mo also bound HF and SF activities (Table 5). One of the MoAbs to DR-α chain (TAL.IB5) adsorbed HF and SF activities but failed to inhibit SA binding to T8+ cells and Mo. The other 2 MoAb which inhibited SA binding to T8+ cells and Mo had no effect on HF or SF activity (OK.Ial, DA6.164). The MoAb to SB2,3 also failed to recognize HF or SF. It is evident that inhibition of antigen binding to T8+ cells and Mo and adsorption of HF or SF activity varies with the McAb to different MHC class II antigens.

A comparative analysis of inhibition of ^{125}I-SA binding to T8+ SC and Mo and adsorption of HF and SF activities with nine MoAbs to HLADR, DC1 and SB2,3 suggests four categories of recognition of class II antigenic determinants (Table 6). (1) Class II determinants common to the antigen binding function of T8+ cells and Mo, and to the HF and SF activities are recognized by the MoAb to DR-β chains (DA6.231) and to DC1. (2) Class II determinants shared only by the antigen binding T8+ cells and Mo are recognized by MoAb to DR-β chains (DA6.164) and to the DR monomorphic determinant (OK.Ial). (3) Class II determinants shared by HF and SF are recognized by the MoAb to DR-α chains (TAL IB5). (4) Class II determinants not recognized by any of the 4 assays; MoAb to DR-α chain, (DA6.147), DR-β chain (HIG.48, HIG.78) and to SB.2, 3 (IL RI).

As both HF and SF have specific antigen binding sites (Lehner, 1983b), it seems that at least one DR-β chain monomorphic determinant and one DC1 determinant is shared by two antigen binding cells and two soluble factors. MHC class II antigenic determinants might therefore play a significant part in antigen binding to cells and in the functions of HF and SF.

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