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Characterization of human helper and suppressor factors with special reference to HLA-DR determinants

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Summary. Human helper and suppressor cells can be induced entirely *in vitro*, by culturing lymphocytes with a small or large dose of antigen for 4 days in a Marbrook–Diener system. The corresponding helper or suppressor factor (*vitro*) can then be released by a second pulse of antigen for 1 day. However, if sensitization has taken place *in vivo* by a naturally encountered antigen, then helper or suppressor factor (*vivo/vitro*) can be released by a single pulse of antigen for 1 day from putative *in vivo*-induced helper or suppressor cells. Helper factor$_{vitro}$ was compared with helper factor$_{vivo/vitro}$, stimulated by a streptococcal protein antigen derived from *Streptococcus mutans*. The results of affinity chromatography suggest that both helper factors have an antigen-specific binding site and bind to monoclonal antibodies directed against HLA-DR, MT, β_2M and μ chain and a 'constant' part on helper factor (HF) determinants. The HF$_{vivo/vitro}$ unlike HF$_{vitro}$ may reflect *in vivo*-sensitization and thus assay putative *in vivo*-sensitized antigen specific helper cells. Similarly, suppressor factor $_{vivo/vitro}$ might be a measure of *in vivo*-induced suppressor cells. Like HF, suppressor factor (SF) has an antigen-specific binding site, HLA-DR, MT and β_2M determinants and a 'constant' portion of SF. The DR determinants were further characterized by immunoadsorption, using monoclonal antibodies to the α- and β-chain of DR antigen. The results suggest that helper and suppressor factor$_{vivo/vitro}$ express similar Ia determinants. Both expressed an α-chain determinant, recognized by one of the two anti-α-chain antibodies (TAL/1B5), and a β-chain non-polymorphic determinant recognized by one of the four anti-β chain antibodies used (DA6.231). However, both HF and SF derived from DRw6- but not from DR4-typed lymphocytes expressed MT1 (DRw6,1,2) or MT2 (DRw6,3,5,8) determinants. As DR and MT determinants can be expressed on the same molecule, it is possible that both are involved in helper and suppressor activities.

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INTRODUCTION

Helper and suppressor cells and factors are involved in a complex regulation of the immune response in primates. Antigen-specific T helper cells and factors are essential for an effective B-cell response in man (Geha, Mudawwar & Schneeberger, 1977; Howie, Feldman, Mozes & Maurer, 1977; Dosch & Gelfand, 1977; Heijnen, Uytdehaag, Dollekamp & Ballieux, 1979; Lamb, Welsh & Batchelor, 1981) and in rhesus monkeys (Lamb, Kontiainen & Lehner 1980; Lehner, Lamb & Kontiainen, 1982). Furthermore, antigen-specific T suppressor cells and factors have also been described in man (Uytdehaag, Heijnen & Ballieux,

1978; Kontiainen, Woody, Rees & Feldmann, 1981; Lehner, 1982) and in rhesus monkeys (Lamb, Kontiainen and & Lehner, 1979; Lehner *et al.*, 1982).

Although helper and suppressor cells and factors have been largely studied in isolation, their co-existence has now been established in man (Lehner, 1982). A dose-related reciprocal relationship was found between the helper and suppressor functions of human lymphocytes. Furthermore, a differential dose-response of about 1000 ng of a streptococcal antigen (SA) was found predominantly between the helper functions of DRw6- and DR4-typed lymphocytes (Lehner, *et al.*, 1981).

The aim of this paper is to characterize by affinity chromatography two types of helper factor (HF). HF_{vitro} was prepared entirely *in vitro* by culturing lymphocytes with a small dose of SA for 4 days to induce helper cells, followed by 1 day culture to release HF. This was compared with $HF_{vivo/vitro}$ which was prepared by culturing putative *in vivo*-induced helper cells with SA for only 1 day, on the assumption that helper cells might have been induced naturally by *Streptococcus mutans*, which is commonly found in the oral cavity of man (Lehner, 1982). Similarly, suppressor $factor_{vitro}$ can be prepared by culturing lymphocytes with a high dose of SA for 4 days, followed by 1-day culture to release the suppressor factor (SF). The corresponding $SF_{vivo/vitro}$ was prepared by releasing the SF from putative *in vivo*-induced suppressor cells. HF and $SF_{vivo/vitro}$ were compared with reference to the presence of immunoglobulin isotypes, class 1 and especially class 2 HLA gene products.

MATERIALS AND METHODS

Preparation and assay of helper cells and factors in vitro
Twelve subjects were used, six of whom had HLA-DRw6 and six had DR4 as one of the DR allotypes, determined previously by standard methods (Welsh & Batchelor, 1978; Lehner *et al.*, 1981). Ficoll-Isopaque density gradient was used to separate peripheral blood lymphocytes (Boyum, 1968). Helper cells were prepared by culturing 5×10^6 viable cells in 1 ml of tissue culture medium, with a pre-determined optimal dose of 10 ng of SA *in vitro* in Marbrook–Diener flasks for 4 days (Lehner, 1982). The cells were harvested, washed and restimulated with 100 ng/ml of SA for 24 hr. The resulting supernatant, containing the putative HF, was collected and tested at a previously determined optimum concentration of 10^{-3} ml per culture in Costar plates (Cambridge, Mass.), in the presence of 10×10^6 unprimed CBA spleen cells and 100 ng/ml of dinitrophenylated SA. The anti-dinitrophenyl (DNP) antibody forming cells (AFC) were assayed on day 4, using the modified Cunningham assay (Cunningham & Szenberg, 1968), with DNP-Fab coated sheep red blood cells (SRBC) and uncoated SRBC. DNP-specific plaques were the difference between the two. Since unprimed spleen cells were used, only IgM AFC were detected. All cultures were carried out in triplicate and assayed separately.

Preparation of factors in vitro *from putative helper and suppressor cells* in vivo
In order to induce HF *in vitro* from putative helper cells (HC) *in vivo* (Lehner *et al.*, 1981), separated lymphocytes were pulsed with a previously determined dose of 1 ng of SA for DRw6 and 1000 ng for DR4 lymphocytes for only 24 hr and any HF released into the supernatant was assayed as described above for AFC. SF was prepared from putative suppressor cells, assumed to be induced *in vivo*, in the same way as for HF. The cells were cultured in the presence of 1 ng SA for DR4 and 1000 ng for DRw6 lymphocytes for 24 hr and the supernatants were then assayed for their ability to suppress helper cells (Lamb *et al.*, 1979). Helper cells were induced from CBA mouse spleen cells by culturing these *in vitro* with 100 ng of SA for 4 days (Lamb *et al.*, 1979). The SF was then cultured in the presence of 3×10^5 HC, 10×10^6 unprimed CBA spleen cells and 100 ng of DNP-SA. AFC were assayed on day 4 as for HF.

Preparation of antigens
Streptococcal antigen I/II (SA) was prepared from *S. mutans* (serotype c, Guy's strain), grown in a semi-defined medium, as described previously (Russell & Lehner, 1978; Russell, Bergmeier, Zanders & Lehner, 1980). Briefly, the culture supernatant was precipitated by ammonium sulphate and chromatographed on DEAE-cellulose, followed by gel-filtration on Sepharose 6B (Pharmacia, Uppsala, Sweden). The resultant protein showed a single band on polyacrylamide-gel electrophoresis and had a molecular weight of about 185,000 (Russell *et al.*, 1980). Keyhole limpet haemocyanin (KLH) was kindly donated by Dr M. Ritterberg of Portland, Oregon. DNP-SA was prepared using dinitrofluorobenzene as described before (Lamb *et al.*, 1979) and had 5 groups of DNP per 100,000 mol. wt.; trinitrophenylated (TNP) KLH was prepared with 8 groups of TNP per 100,000 mol. wt.

Preparation and use of immunoadsorbents

The antisera or antigens were coupled to cyanogen-bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) at a concentration of 100–500 μl of the antiserum or 1 mg of antigen per ml of beads. The coupling reaction was carried out at room temperature for 2 hr and terminated with 0·1 methanolamine pH 9·0, followed by washing with bicarbonate buffer pH 8. HF was diluted 1 : 10 and then added to the beads in the ratio of 1 ml of factor to 1 ml of beads. These were then mixed for 2 hr at 4° and the unbound material was recovered by centrifugation, millipore filtered and stored at −20° until further use. The beads were washed three times with phosphate-buffered saline (PBS) and the bound material eluted from the beads by washing with 3 M sodium thiocyanate (British Drug House), in the ratio of 1 ml per ml of beads. The eluted material was diluted with an equal volume of PBS, dialysed against saline for 24 hr, millipore filtered and stored at −2·0°.

Two groups of antisera were used. (i) Rabbit antisera to human IgG, IgM, IgA and IgD (Behringwerke Ltd.) were used, at a concentration of 1 mg per ml beads. A monoclonal anti-IgM antibody (100 μl; Bethesda Research Laboratories) was further used to confirm the findings with the rabbit anti-IgM. Anti-SA antiserum was raised in rabbits as described previously (Russell *et al.*, 1980). Anti-HF and anti-SF antisera were prepared in rabbits and kindly donated by Dr S. Kontiainen (Kontiainen & Feldmann, 1979). (ii) Monoclonal antibodies to various HLA determinants were: anti-HLA-A,B (2A1) at 500 μl per ml beads, (kindly supplied by Dr P. C. L. Beverley; Beverley, 1979); anti-human β_2M at 1 mg per ml beads (kindly provided by Dr A. Sanderson; Trucco, Stocker & Ceppellini, 1978); monomorphic HLA-DR antibodies (DA2) and the anti-HLA-DRw6,1,2 or MT1 antibodies (Genox 353 and SDR 1.2) used at 200 μl per ml beads, (kindly provided by Dr W. F. Bodmer and Dr M. J. Crumpton; Brodsky, Parham, Barnstable, Crumpton & Bodmer, 1980; De Kretser, Crumpton, Bodmer & Bodmer, 1982). Anti-HLA-DRw6,3,5,8 or MT2 antiserum (8w 1247) (Trucco *et al.*, 1978), used at 200 μl per ml beads, was provided by Dr D. Murray (Sera-lab No. MAS 044). A monoclonal antibody recognizing null cells (H.25) was also generously provided by Dr W. F. Bodmer and Dr Y. Bai and the above volumes were used. Further monoclonal antibodies to the human Ia-like antigens were kindly supplied by Mr K. Guy, and 500 μl per ml beads were used. DA6.231, DA6.147, HIG.48 and HIG.78 recognize distinct non-polymorphic determinants of human Ia-like antigens (Guy, van Heyningen, Cohen, Deane, Crichton & Steel, 1982; Guy, van Heyningen, Cohen, Deane & Steel, 1982; Steel, van Heyningen, Guy, Cohen, Deane, Crichton & Hutchins, 1982). DA6.231, HIG.48 and HIG.78 react with isolated β-chains and DA6.147 with isolated α-chains. DA6.231 inhibits SB- as well as DR-specific T-cell killing (Shaw S., unpublished). However, DA6.164 recognizes a polymorphic Ia β-chain determinant which is absent from DR-7 homozygous cells (Guy *et al.*, 1982a). Another antibody reacting with an α-chain determinant (Tal/1B5) was provided by Dr W. F. Bodmer and Dr T. E. Adams, and 200 μl per ml beads were used (Adams, Bodmer and Bodmer, in preparation).

RESULTS

A comparative characterization of HF_{vitro} and $HF_{vivo/vitro}$ by immunoadsorption with specific antisera and antigens

The HF that was prepared by first culturing lymphocytes with antigen for 4 days in order to induce helper cells, followed by further stimulation with antigen for 1 day to relase HF, will be referred to as HF_{vitro}, i.e. both the putative helper cell and factor were induced *in vitro*. On the other hand, *in vivo*-sensitized putative helper cells which were stimulated with antigen *in vitro* only for 1 day will be termed $HF_{vivo/vitro}$. Antigen specificity of both helper factors is evident from the factors binding to SA but not to the KLH-coated sepharose beads (Table 1). This suggests a specific SA binding site on the HF. However, whilst free antigen seems to be present in HF_{vitro} this has not been found in $HF_{vivo/vitro}$ on adsorption with anti-SA antiserum. Similarly, anti-HLA (2A1) and anti-Ia (DA2) antibodies bound only HF_{vitro} and not $HF_{vivo/vitro}$, though anti-β_2M bound both HF (Table 2). This suggests that β_2M is an essential component in both factors but that the anti-HLA-A,B and D framework antibodies are not integral parts of helper activity. However, the monoclonal antibody to DRw6,1,2 (Genox 353) absorbed out all the helper activity from DRw6 lymphocytes, without any effect on the helper activity from DR4 lymphocytes, irrespective of whether HF_{vitro} or $HF_{vivo/vitro}$ was tested.

Both factors bound to the anti-HF but not to the anti-SF antisera, thus showing specificity of the 'constant' region of HF (Table 1). Adsorption with anti-IgG, IgA or IgD bound neither HF. However, half of the HF_{vitro} and $HF_{vivo/vitro}$ bound the rabbit

Table 1. Comparison of immunoadsorption of helper factor$_{vitro}$ with helper factor$_{vivo/vitro}$ for specificity, immunoglobulin and 'constant' region determinants; the results are given as the mean ±SEM of antibody-forming cells

Absorbent	No. of specimens	Helper factor$_{vitro}$		No. of specimens	Helper factor$_{vivo/vitro}$	
		Bound	Not bound		Bound	Not bound
Nil (DNP-SA)	7	20·1±2·7		9	25·2±2·1	
Nil (DNP-SA+HF)	7	145·9±6·7		9	142·6±10·8	
SA	4	105·5±4·8	25·8±3·6	4	97·5±7·2	22·3±7·4
KLH	4	18·2±2·1	140±7·6	4	38·2±7·3	191·5±6·8
SA antiserum	4	145·0±7·9	28·2±7·9	6	36·8±7·2	119·8±7·0
HF antiserum	4	125·8±6·8	11·0±4·6	4	140·5±9·4	23·2±4·9
SF antiserum	4	25·0±5·8	120·8±9·2	4	15·0±6·2	110·8±9·0
IgG antiserum	4	15·2±8·0	110·8±4·5	4	21·5±0·9	113·2±8·6
IgA antiserum	4	21·0±5·6	122·5±3·8	4	14·8±9·1	113·8±4·2
IgD antiserum	4	28·2±5·4	109·0±14·7	4	14·8±2·8	107·2±13·9
IgM antiserum	4	70·8±17·9	63·2±23·9	4	76·8±19·1	89·8±23·1
IgM* antiserum	4	133·3±18·3	20·3±3·3	4	107·5±1·7	31·8±9·6

* Monoclonal antibody

Table 2. Comparison of immunoadsorption of helper factor$_{vitro}$ with helper factor $_{vivo/vitro}$ for HLA antigens; the results are given as the mean ±SEM of antibody forming cells

Absorbent	No. of specimens	Helper factor$_{vitro}$		No. of specimens	Helper factor$_{vivo/vitro}$	
		Bound	Not bound		Bound	Not bound
Nil (DNP-SA)	7	20·1±2·7		9	25·2±2·1	
Nil (DNP-SA+HF)	7	145·9±6·7		9	142·6±10·8	
Monoclonal antibodies:						
HLA (framework) (2A1)	4	111·5±4·7	22·5±4·6	4	18·5±3·5	107·2±3·6
β_2M	5	150·0±12·1	31·2±6·7	5	115·4±7·1	37·2±13·5
Null cell (H.25)		ND	ND	5	32·9±4·4	202·4±13·4
Ia framework (DA2)	7	130·0±16·0	39·7±7·4	8	40·9±5·5	122·9±9·4
MT1; DRw6,1,2						
DRw6 lymphocytes	3	153·7±14·5	29·0 5·9	6	119·0±15·1	38·2±8·6
DR4 lymphocytes	3	48·0±5·9	122·3±7·9	5	23·8±8·2	109·2±4·3

ND Not done

anti-human IgM, though when monoclonal anti-IgM was used all eight HF$_{vitro}$ and HF$_{vivo/vitro}$ were bound, suggesting that a part of the μ-chain or some cross-reactive component may be required for HF activity. The null cell determinant recognized by the monoclonal antibody H.25 failed to bind HF$_{vivo/vitro}$ (Table 2).

Characterization of SF$_{vivo/vitro}$ by immunoadsorption

SF released by stimulation of *in vivo*-sensitized human putative suppressor cells with SA for 1 day showed similar characteristics to HF$_{vivo/vitro}$ (Table 3). SF binds to SA but not to KLH-coated sepharose beads. The factor also binds to β_2M but not to HLA antibodies or to SA antiserum. However, unlike HF$_{vivo/vitro}$, SF$_{vivo/vitro}$ does not bind to IGM antiserum and binds to SF but not HF antiserum. Anti-DRw6,1,2 antibodies bind factor from DRw6- but not DR4-stimulated lymphocytes (Table 5).

Table 3. Immunoadsorption of suppressor factor$_{vivo/vitro}$ with antigens and antibodies; the results are given as the mean ±SEM of antibody forming cells

Absorbent	No. of specimens	Suppressor factor$_{vivo/vitro}$ Bound	Not bound
Nil (DNP-SA)	12	28·3±1·7	
Nil (DNP-SA+HC)*	12	252·8±13·1	
Nil (DNP-SA+HC+SF)	22	36·7+2·5	
Streptococcal antigen	6	28·3±5·2	208·7±10·1
KLH	6	216·7±3·8	34·8±3·2
SA antiserum	6	164·5±11·5	21·7±4·2
Helper factor antiserum	6	184·7±17·0	41·0±3·4
Suppressor factor antiserum	6	31·7±4·0	191·2±13·6
IgG antiserum	6	159·5±8·7	39·8±6·0
IgA antiserum	6	186·2±7·5	43·2±7·6
IgM antiserum	6	133·3±10·0	42·0±5·4
HLA (framework) Antibodies	6	167·8±16·8	51·2±12·8
β_2M Antibodies	6	32·0±5·6	165·7±18·4
Null Cell Antibody (H.25)	3	203·3±10·8	42·3±2·9

* HC = Helper Cell (Mouse)

A comparative characterization of helper and suppressor factors (*vivo/vitro*) by immunoadsorption with monoclonal antibodies to HLA DR and MT determinants

Both factors failed to be absorbed by antibodies to Ia-framework (DA2), β-chain polymorphic (DA6.164) and β-chain non-polymorphic antibodies (HIG.48 and HIG.78) (Table 4). HF and SF were absorbed by the monoclonal antibodies to B-chain framework (DA6.231) which also recognizes SB determinants. SB (secondary B cell) antigens are encoded by a HLA-linked locus which is distinguished from HLA-DR antigens (Shaw *et al.*, 1981). The two α-chain antibodies behaved rather differently; whereas TAL/1B5 bound both HF and SF, DA6.147 bound neither factor. However, the two MT1 and the MT2 antibodies showed consistent results (Table 5). Factors from DRw6 unlike DR4 lymphocytes were absorbed by both the MT1 or anti-DRw6,1,2 antibodies (Genox 353 and SDR1.2) and the MT2 or anti-DRw6,3,5,8 (8w 1247) antibodies.

Table 4. Comparison of immunoadsorption of helper and suppressor factors$_{vivo/vitro}$ with monoclonal antibodies to Ia determinants; the results are given as the mean ±SEM of antibody forming cells

Monoclonal antibodies	No. of specimens	Helper factor$_{vivo/vitro}$ Bound	Not bound	No. of specimens	Suppressor factor$_{vivo/vitro}$ Bound	Not bound
Nil (DNP-SA)	9	35·8±3·8		9	27·4±2·5	
Nil (DNP-SA+HF)	9	197·3±10·1				
Nil (DNP-SA+HC)				9	253·7±11·4	
Nil (DNP-SA+HC:SF)				9	44·1±5·4	
Ia framework (DA2)	8	40·9±5·5	122·8±9·4	6	179·3±17·4	42·8±9·3
α-chain (DA6.147)	8	28·8±4·4	136·4±15·0	6	186·0±12·9	26·0±7·7
α-chain (TAL/1B5)	6	185·0±11·2	29·5±3·1	3	45·3±6·2	221·3±20·2
β-chain polymorphic (DA6.164)	6	50·7±10·1	158·2±27·3	6	193·3±6·3	44·8±5·6
β-chain framework (DA6.231) (SD reactivity)	4	177·2±14·8	36·0±7·8	6	35·3±6·4	178·3±15·5
β-chain (HIG.48)	3	32·0±9·5	157·7±22·6	3	177·7±3·9	57·7±9·6
β-chain (HIG.78)	3	59·0±18·0	123·3±8·2	3	148·7±14·3	54·3±7·2

Table 5. Comparison of immunoadsorption of helper and suppressor factors$_{vivo/vitro}$ with monoclonal antibodies to MT antigens; the results are given as the mean ±SEM of antibody forming cells

Monoclonal antibodies	No. of specimens	Helper factor$_{vivo/vitro}$ Bound	Helper factor$_{vivo/vitro}$ Not bound	No. of specimens	Suppressor factor$_{vivo/vitro}$ Bound	Suppressor factor$_{vivo/vitro}$ Not bound
Nil (DNP-SA)	15	42·3±3·4		15	38·7±4·1	
Nil (DNP-SA+HF)	15	211·4±9·6				
Nil (DNP-SA+HC)				15	238·1±8·4	
Nil (DNP-SA+HC+HF)				15	45·3±3·6	
MT1: DRw6,1,2 (Genox 353)						
DRw6 lymphocytes	6	119·0±15·1	38·2±8·6	3	36·7±7·1	175·3±27·0
DR4 lymphocytes	5	23·8±8·2	109·2±4·3	3	158·0±21·8	23·3±2·0
MT1: DRw6,1,2 (SDR.1)						
DRw6 lymphocytes	3	175·7±30·6	45·7±5·7	3	43·3±5·2	200·0±15·3
DR4 lymphocytes	3	44·7±2·3	197·7±11·8	3	206·7±11·7	36·7±9·5
MT2: DRw6,3,5,8 (8w 1247)						
DRw6 lymphocytes	3	151·3±14·3	71·0±4·2	3	40·0±5·1	140·0±16·6
DR4 lymphocytes	3	80·0±23·8	178·3±19·6	3	133·3±27·1	57·7±12·7

DISCUSSION

Helper cells induced *in vitro* by stimulation with a streptococcal antigen for 4 days release helper factor activity on further stimulation with antigen for 1 day (HF_{vitro}; Lehner, 1982). There is no difference in response between the cells derived from HLA-DRw6 and those from DR4-typed subjects, as all of them respond to 10 ng of the SA. This is clearly different from the assay of putative *in vivo*-induced helper cells, for DRw6 cells release HF after stimulation with 1–10 ng of SA for 1 day and DR4 cells require 1000 ng for the release of a corresponding amount of HF ($HF_{vivo/vitro}$). Thus, $HF_{vivo/vitro}$ measures putative *in vivo*-induced HC and may reflect the helper status to specific antigens in man, whereas HF_{vitro} gives only the capacity of lymphocytes to be sensitized *in vitro* to helper cell and factor activity.

Comparative adsorption studies of the two HF have shown that $HF_{vivo/vitro}$, unlike HF_{vitro}, does not bind to antisera against the SA, HLA-A,B and DR framework antigens (Tables 1, 2). It is likely that culture of lymphocytes *in vitro* for 4 days activates the cells to release a number of membrane antigens. These antigens and the added SA may adhere non-specifically to the HF and give false reactions or are not involved in the functional manifestations of these factors. This is less likely to occur with $HF_{vivo/vitro}$ in which the putative helper cells have already been induced under physiological conditions *in vivo* and only one pulse of antigen is used for 1 day to release the HF. The same interpretation might apply to SF_{vitro} and $SF_{vivo/vitro}$, but the former has not been characterized in man. Thus, $HF_{vivo/vitro}$ and, most likely, $SF_{vivo/vitro}$ are cleaner preparations than HF_{vitro} and SF_{vitro}. This view is supported by a three-stage purification of HF_{vitro} from human spleen cells by affinity chromatography on a column of SA bound to Sepharose; the bound material was eluted and passed through a second column of a monoclonal antibody to human β_2M and the bound material was then eluted and further purified by gel filtration on Sephadex G75, using only one fraction which co-eluted with human serum albumin and which retained all the helper activity (Zanders E.D., in preparation). This triple-purified HF_{vitro}, unlike the unfractionated HF (Zanders, Lamb, Kontiainen & Lehner, 1980) failed to bind to anti-SA and anti-HLA-A,B antisera, suggesting that these antigens are not integral parts of HF. Nevertheless, the possibility that HF_{vitro} and $HF_{vivo/vitro}$ are two different factors cannot be dismissed.

A comparison of the components of HF with those of SF (*vivo/vitro*) showed that they have an antigen-specific binding site (Tables 1, 3), as has been found in other human HF (Heijnen *et al.*, 1979; Zvaifler, Feldmann, Howie, Woody, Ahmed & Hartzman, 1979; Mudawwar, Yunis & Geha, 1978), and SF (Uytedehaag *et al.*, 1978; Kontiainen *et al.*, 1981). β_2M was found in both HF and SF, unlike the HLA-A,B framework determinant. Indeed, β_2M was also reported in monkey HF_{vitro} with three out of four anti-human β_2M antisera, raised in four species of animals and by a monoclonal anti-β_2M (Lamb, Zanders, Sanderson, Ward, Feldmann, Kontiainen,

Lehner & Woody, 1981). β_2M was also detected in the allogeneic effect factor (Armerding, Kubo, Grey & Katz, 1975) and in an IgE suppressor factor (Katz & Tung, 1979). The presence of a 'constant' region of HF, as defined by Feldmann, James, Culbert, Todd, Makidono, Cecka & Kontiainen, (1981) was found in HF but not SF, and a 'constant' region of SF was found in SF and not HF. These results are consistent with finding a 'constant' region of HF but not of SF in monkey HF_{vitro} (Zanders *et al.*, 1980) and the 'constant' region of SF_{vitro} in man (Kontiainen *et al.*, 1981). A μ-chain determinant, however, was detected only in HF and it is of interest that the ambiguous results with polyclonal anti-IgM antiserum were resolved by using monoclonal anti-IgM antibody, showing a μ-chain or a cross-reactive determinant in $HF_{vivo/vitro}$. This has also been found in monkey HF_{vitro} (Zanders *et al.*, 1980) and in mouse HF_{vitro} (Feldmann & Basten, 1972).

Analysis of the immunoadsorption data with the monoclonal anti-HLA antibodies showed that helper and suppressor factors were not absorbed by the anti-HLA-A,B and DR framework antibodies, suggesting that they may lack the determinants recognized by these antibodies. Of the two α-chain antibodies, the TAL/ B5 absorbed whereas the DA6.147 failed to absorb the HF and SF. Although this may seem surprising, it now appears that there are three types of α-chains (α1, α2 and α3) so that the α-chain antibodies might be directed to different types or determinants of α-chains. Absorption with a number of monoclonal antibodies directed to β-chain determinants (Guy *et al.*, 1982a, b) revealed that the polymorphic anti-β-chain antibody (DA6.164) and the non-polymorphic anti-β-chain antibodies (HIG.48 and HIG.78) failed to absorb the HF or SF activities (Table 4). However, the anti-β-chain framework antibody (DA6.231), which also recognizes SD determinants, absorbed both HF and SF, irrespective of the DR type of lymphocyte. These results suggest that the factors might have either a β-chain non-polymorphic determinant or a SB determinant or both.

Furthermore, factors derived from DRw6-, unlike those from DR4-typed lymphocytes were absorbed by the two MT1 (DRw6,1,2) and the MT2 (DRw6,3,5,8) antibodies. These findings argue in favour of MT antigens playing a part in the functions of HF and SF. The presence of MT in addition to DR antigens need not be surprising, since there is considerable evidence that homozygous cell lines may express both DR and MT determinants on the same molecule (Mann, 1979; Karr, Kannapell, Stein, Gebel, Mann, Duquesnoy, Fuller, Rodey & Schwartz, 1982; Markert & Cresswell, 1982). The relationship between DR, MT and SB antigens is as yet unclear but recent evidence suggests that there exist three β-chains (De Kretser, Crumpton, Bodmer & Bodmer, 1982). β1 corresponds to the DR polymorphic antigens, β3 to MT antigens and β2 might represent SB antigens. The absorption data suggest that the MT antigens are well expressed in both factors but it is difficult to be confident about the DR antigens. The lack of monoclonal antibodies to the polymorphic DR antigens (β1) makes it difficult to establish if these antigens are expressed in HF and SF. However, absorption with the α-chain antibody (TAL/1B5) and β-chain framework antibody (DA6.231), although not with the polymorphic β-chain antibody (DA6.164) or non-polymorphic β-chain antibodies (HIG.48 and HIG.78), argues in favour of HF and SF bearing some expression of the DR gene locus. Nevertheless, a great deal of further investigation will be required before any definitive conclusions can be drawn about the functional relevance of DR, MT and SB determinants in helper and suppressor activities.

It is difficult to envisage that HF which has a molecular weight of about 70,000 (as determined in the monkey HF_{vitro} (Zanders *et al.*, 1980) can incorporate the above components. It is possible that this may be part of an immunoglobulin-like molecule and that only the functional determinant of each antigen needs to be present. Whilst we could indulge in a great deal of speculation, this problem will be eventually elucidated by characterization and examination of the homology of amino-acid sequences of pure HF and SF from corresponding T-cell clones.

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