Immunology 1989 **68** 147–153

The selective antigen-presenting cell capacity of activated B lymphocytes in HLA-II-restricted responses of CD4$^+$ T lymphocytes

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Accepted for publication 26 June 1989

SUMMARY

We examined the role of antigen-presenting B lymphocytes using panels of antigen-specific CD4$^+$8$^-$ T-lymphocyte clones (TLC). All 19 TLC showed a class II major histocompatibility complex-encoded (HLA-II) restricted proliferation to antigen presented by antigen-presenting cells (APC) from the monocyte fraction of peripheral blood. Only six TLC were effectively activated by antigen presented by autologous B lymphocytes activated by EBV transformation. This failure of B lymphocytes was not due to: (i) a high degree of cell surface sialic acid; (ii) a low expression of the cell surface proteins HLA-II, ICAM-1 or LFA-3 that restrict antigen presentation; (iii) lack of secretion of the cytokine IL-1 or other soluble factors that may be required as secondary signals; or (iv) induction of incomplete T-cell activation resulting in the production of growth factor interleukin-2 (IL-2) or the expression of receptors for IL-2 only. These data suggest the involvement of another cell surface interaction in antigen presentation acting besides the interactions known so far.

INTRODUCTION

T cells can be stimulated to proliferation as a result of the specific interaction of the T-cell receptor complex with antigen in association with class II major histocompatibility complex (HLA-II) molecules present on the cell surface of antigen-presenting cells (APC) (Benacerraf, 1978). To accomplish this antigen-specific recognition, APC and T cells adhere by bridging of CD2-LFA-3 and LFA1–ICAM1 (Davignon *et al.*, 1980; Dougherty, Murdoch & Hogg, 1988; Bierer *et al.*, 1988). Most antigens require processing steps for T-cell activation, whereas some antigens do not (Allan, 1987). The second signal in T-cell activation is provided by non-specific factors, at least one of which is IL-1, liberated by the APC (Scala & Oppenheim, 1983). A whole variety of cells that have a constitutive or inducible expression of HLA-II molecules can present antigen to T cells. These include dendritic cells (Steinman & Nussenzweig, 1980), monocytes (Bergholtz & Thorsby, 1979) and macrophages (Rosenthal & Shevach, 1973), endothelial cells (Hirschberg, Braathen & Thorsby, 1982), fibroblasts (Geppert & Lipsky, 1985), B lymphocytes (Chesnut & Grey, 1981; Glimcher *et al.*, 1982; Ashwell *et al.*, 1984; Lanzavecchia, 1985) and activated T lymphocytes (Brown, Cook & Rich, 1987).

Some recent investigations (Ron & Sprent, 1987; Kurt-Jones *et al.*, 1988) are indicative for a crucial role of B cells in the *in vivo* induction of T-cell help. Previous *in vitro* studies showed that the antigen-presenting capacity of B cells resides in B cells activated by lipopolysaccharide (LPS) or Epstein–Barr virus (EBV) transformation, since resting B are less effective APC (Frohman & Cowing, 1985; Zlotnik *et al.*, 1983; Metlay, Pure & Steinman, 1989). The poor antigen-presenting capacity of resting B cells can be enhanced by treatment with neuraminidase (Frohman & Cowing, 1985) or by incubation with IL-4 (Zlotnik *et al.*, 1987).

However, the crucial *in vivo* role of activated B cells as APC contrast with *in vitro* studies that have shown that human and murine B cells are less effective APC than adherent cells from peripheral blood or spleen (Hanke *et al.*, 1987; Dekruyff, Cantor & Dorf, 1985; Inaba & Steinman, 1984; Metley *et al.*, 1989). In agreement with this, we found that the majority of antigen-specific human TLC that respond to antigen presented by APC from peripheral blood did not respond *in vitro* to soluble antigen presented by activated B.

In the present study we have attempted to elucidate the mechanisms that underly the defective antigen-presenting capacity of B cells using cloned antigen-specific T cells, with an emphasis on TLC that specifically proliferate to nickel ions. The advantage of this model is that, like for most allergenic haptens (Clement & Shevach, 1979; Forman *et al.*, 1979), nickel does not

Abbreviations: APC, antigen-presenting cell; CA, *Candida albicans*; CD, cluster of differentiation; D.pt. *Dermatophagoides pterinyssinus*; EBV-B, Epstein–Barr virus-transformed B cells; HLA, human leucocyte antigen; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; LFA, lymphocyte functional antigen; Ni, nickel sulphate; PBMC, peripheral blood mononuclear cells; TcR, T-cell receptor; TLC, T-lymphocyte clone; TT, tetanus toxine.

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require intracellular antigen processing (Kapsenberg *et al.*, 1988a). With the use of these TLC a possible role for differential antigen processing by different APC subsets was avoided.

MATERIALS AND METHODS

Antibodies and reagents

The following antibodies were used: anti-CD4 from Dako (Glostrup, Denmark); anti-4B4 (anti-CD45R) and anti-2H4 (anti-CD29) from Coulterclone (Hialeah, FL); OKT3 (anti-CD3), OKT8 (anti-CD8) and OKM1 (anti-CD11b) from Ortho (Raritan, NJ); Leu 16 (anti-CD20) from Beckton-Dickinson (Mountain View, CA); CLB IL-2R (anti-CD25) from the Central Laboratory Blood Transfusion Service (Amsterdam, The Netherlands); PdV 52 (anti-HLA-II) and B8.11.2 (anti-HLA-DR) were a kind gift from Dr F. Koning (Dept. Immunohematology, University of Leiden); SPV-L-3 (anti-HLA-DQ) was a kind gift of Dr H. Spits (Cancer Institute, Amsterdam); RR 1/1 (anti-ICAM1) and ST 2/9 (anti-LFA3) were a kind gift from Dr T. Springer (Dana Farber Cancer Institute, Boston).

The antigens were: $NiSO_4 \times 6H_2O$ (Ni), obtained from Merck (Darmstadt, FRG); purified protein antigens from housedust mite *Dermatophagoides pteronyssinus* (D.pt.) and *Candida albicans* (CA) from ARTU biologicals (Lelystad); and tetanus toxine (TT), a kind gift from Dr F. Uytenhaag (RIVM, Bilthoven).

Recombinant proteins used were: interleukin-1β (IL-1β) from Biogen (Geneva, Switzerland); interferon-gamma (IFN-γ), a kind gift from Dr P. van der Meide (TNO, Rijswijk); interleukin-6 (IL-6), a kind gift from Dr L. Aarden (Central Laboratory of Blood Transfusion Service); interleukin-2 (IL-2) from Cetus (Emeryville, CA); interleukin-4 (IL-4), a kind gift from Dr J. E. de Vries (UNICET, Dardilly, France).

Determination of antigen expression

Cell typing was performed on cytospin preparations with double-layer staining using FITC-conjugated and Fab-fragmented affinity-purified rabbit anti-mouse Ig (Zymed, San Francisco, CA) as a second layer. Expressions of antigens were determined microscopically or cytofluorimetrically using the same labelling procedure as for cell typing.

T lymphocyte clones (TLC)

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Ficoll-metrizoate density centrifugation at density = 1·077 g/ml. 2×10^5 PBMC were cultured in 200 μl culture medium consisting of Iscove's modified Dulbecco's medium (IMDM) (Gibco's Laboratories, Paisley, Renfrewshire, U.K.) supplemented with 10% pooled human serum (HS), 100 U/ml penicillin and 100 μg/ml streptomycin. T cells were activated by 50 μg/ml D.pt., 1 Lf/ml TT, 10 μg/ml CA or 10^{-4} M Ni. After 4 days of incubation at 37° growth factor 10% IL-2-containing conditioned medium was added. After another 3 days lymphoblasts were enriched by Ficoll-metrizoate density centrifugation at d = 1·066 g/ml. The blasts were cloned at limiting dilution in 96-well flat-bottomed plates (Falcon, Beckton-Dickinson) in the presence of mitogen (1 μl/ml phytohaemagglutinin; Difco, Detroit, MI), 1×10^5 allogenic irradiated (2700 rads) PBMC of unrelated donors, 1×10^4 allogenic irradiated (3300 rads) Epstein–Barr virus transformed B cells (EBV-B) and growth factor (40 U/ml rIL-2 or 10% IL-2-containing conditioned medium). After 10 to 14 days, wells with growing cells were scored and the cells were transferred to 24-well macrowell plates (Falcon) and cultured further with growth factor.

Antigen-presenting cells (APC)

Autologous APC from peripheral blood were isolated as described before (Res *et al.*, 1987). In short: PBMC were allowed to adhere for 90 min at 37° to plastic petri-dishes coated with human serum or fibronectin. Adherent cells were harvested by incubation with PBS supplemented with EDTA. The isolated cells showed a dendritic or monocyte-like morphology and stained for more than 95% with OKM1. To prepare B cells, autologous PBMC were labelled with monoclonal mouse anti-human IgM (MH 15-01-M02; CLB, Amsterdam). Labelled cells were panned to petri-dishes coated with 3 μg/ml affinity-purified goat anti-mouse Ig (Tago, Burlingame, CA) for 70 min at 4°. More than 98% of the adherent cells were B cells according to their expression of surface Ig, as determined by indirect peroxidase staining on cytospin preparations using polyvalent goat anti-human Ig. In order to prepare autologous EBV-B cells from Donors 1 and 2, cells were depleted of T cells by E rosetting. 1×10^5 non-rosetting cells were cultured in 96-well flat-bottomed microwells in IMDM with 10% fetal calf serum (FCS; Flow Lab., Irvine, Ayrshire, U.K.) containing 50% supernatant of an EBV-producing marmoset cell line. EBV-B stained with Leu 16 (anti-CD20) and were negative for CD3 (OKT3) and CD11b (OKM1).

Cell surface sialic acid was digested by treatment with 100 U/ml neuraminidase (ex Vibrio coma; Koch-Light Ltd, Suffolk, U.K.) for 30 min at 37°.

Pretreatment of EBV-B with cytokines was performed by culturing 2×10^5 EBV-B in 24-well plates for 4 days in the presence of 1000 U/ml rIL-1β, 1000 U/ml rIFN-γ, 5000 U/ml rIL-6 or 200 U/ml rIL-4.

Proliferation assays

TLC were cultured in 200 μl culture medium (IMDM supplemented with HS, penicillin and streptomycin) in 96-well flat-bottomed plates in the presence of 1×10^5 irradiated (2700 rads) PBMC, 2×10^4 purified monocytes or 2×10^4 irradiated (3300 rads) EBV-B in the presence or absence of antigen, as indicated in the cloning protocol. The cells were cultured for 40 hr, the last 16 hr in the presence of 0·5 μCi (18·5 kBq of tritiated thymidine). Proliferation is expressed as the mean c.p.m. with SD.

RESULTS

Antigen-specific TLC

Antigen-specific TLC were prepared from PBMC that were primed *in vitro* 7 days prior to cloning. This relative short priming period was used to prevent the overgrow by fast growing T cells, that would unwillingly result in limited T-cell repertoires. TLC were raised that specifically responded to purified protein from house dust mite D.pt, TT, CA and Ni. All TLC were phenotypically characterized as T cells of the inducer type ($CD3^+4^+8^-$). Furthermore they expressed the activation antigen CDw29 and did not express CD45R. Antigen-specific proliferation of these TLC required the participation of APC (Table 1). Nickel-specific TLC are considered to be specific, since they do not proliferate in the presence of other antigenic

Table 1. Antigen-specific TLC show a functional heterogeneity based on their APC requirements

TLC	Restriction* determinant	Proliferative response (c.p.m. ± SD)† PBMC No Ag	PBMC Ag	EBV-B No Ag	EBV-B Ag
Donor 1					
D.pt.-specific					
MBB.A5	HLA-DR1	70 ± 30	7844 ± 40‡	2845 ± 18	2440 ± 90
A9	HLA-DR3	178 ± 2	21,478 ± 1423	2355 ± 75	5989 ± 1863
A19	HLA-DR1	216 ± 16	12,613 ± 1767	2521 ± 153	2588 ± 153
A21	HLA-DR3	71 ± 8	29,754 ± 1490	2992 ± 65	25,973 ± 1315
TT-specific					
MBB.B9	HLA-DR1	921 ± 268	18,749 ± 542	3228 ± 35	10,928 ± 498
B15	HLA-DR1	431 ± 71	4537 ± 1026	412 ± 70	435 ± 74
B20	HLA-DR1	1105 ± 148	5714 ± 374	2138 ± 85	2151 ± 44
B22	HLA-DR1	1380 ± 477	8042 ± 127	2289 ± 171	3261 ± 337
CA-specific					
MBB.C8	HLA-DR3	156 ± 35	28,883 ± 689	2992 ± 277	2848 ± 100
C16	HLA-DR1	76 ± 13	14,972 ± 941	3141 ± 167	3866 ± 320
Ni-specific					
MBB.D3	HLA-DQw1	118 ± 71	4367 ± 372	1745 ± 436	1879 ± 42
D7	HLA-DR1	67 ± 48	16,739 ± 330	1492 ± 45	2369 ± 128
D17	HLA-DR1	39 ± 15	9183 ± 174	2118 ± 139	18,971 ± 211
Donor 2					
Ni-specific					
TAB.9	HLA-DR*n*1§	28 ± 23	20,957 ± 363	1157 ± 20	40,777 ± 316
19	HLA-DRw11	50 ± 13	6167 ± 1713	1194 ± 89	22,280 ± 124
26	HLA-DQ*n*2	24 ± 8	17,888 ± 923	1012 ± 83	2318 ± 148
31	HLA-DQ*n*4	26 ± 2	17,556 ± 3831	1086 ± 132	1076 ± 162
37	HLA-DQw1	58 ± 1	15,360 ± 1612	999 ± 235	1204 ± 112
38	HLA-DR1	40 ± 2	4011 ± 4	1068 ± 148	6655 ± 839

* Restriction determinants were determined using blocking anti-HLA-sublocus antibodies and panels of differently HLA-typed APC.

† TLC (2×10^4 cells/well) were cultured for 40 hr with 2700 rads irradiated PBMC (2×10^5) or 3300 rads irradiated EBV-B (2×10^4) as APC in the absence or presence of the antigens (Ag) D.pt (50 μg/ml), TT (1 Lf/ml), CA (10 μg/ml) or Ni (10^{-4} M).

‡ Underlined data means an antigen-specific response higher than five times the response without antigen.

§ Unidentified restriction determinants *n*1, *n*2, etc. These unidentified restriction determinants appeared to be mutually different.

metal ions, like palladium, copper or cobalt, or to various other antigens. In addition, TLC that specifically proliferate to other antigens did not proliferate to nickel.

B cells are functional APC for only a minority of the TLC

In order to examine the antigen-presenting capacity of activated B cells we have used autologous EBV-B lines for their ability to present antigen to the TLC. Although all TLC proliferated to antigen presented by PBMC, only six out of 19 TLC tested, responded to antigen presented by EBV-B (Table 1). B cell-responding T-cell clones required 2×10^4 B cells to reach a proliferative response as reached with 1×10^5 peripheral blood APC. Separation of lymphocytes and monocytes showed that the APC from peripheral blood resided in the fraction of the monocytes. B-cell non-responder clone TAB 31 only proliferated in the presence of monocytes and was not able to respond to antigen in the presence of up to 3×10^5 EBV-B (Table 2). B cells did not suppress T-cell proliferation, since no suppression was found when 2×10^4 EBV-B were added to a culture of T cells proliferating to antigen presented by monocytes (data not shown). The possibility was considered that these T cells respond to another antigen concentration when B-cell APC are used. We have varied the nickel sulphate concentration from 10^{-5} to 10^{-3} M, but at none of these nickel concentrations was TLC TAB 31 activated with B-cell APC (Fig. 1).

EBV-B are a relative selection of normal B cells (Moss & Pope, 1972). They may also have changed antigen-presenting capacities as a result of their transformation. These considerations urged a comparison of normal B cells and EBV-B as APC. B-cell responder TAB 19 proliferated equally well to antigen presented by either 1×10^5 B cells or 2×10^4 EBV-B.

Table 2. The relative antigen presenting capacity of adherent and non-adherent peripheral blood cells and EBV-B cells

APC	No. APC	Proliferative response (c.p.m. ± SD)* of TLC TAB 31	
		No nickel	Nickel
PBMC	3×10^5	86 ± 4	30,378 ± 1015
	1×10^5	69 ± 8	12,005 ± 2727
	3×10^4	52 ± 18	1467 ± 437
	1×10^4	42 ± 9	115 ± 13
Adherent fraction	1×10^5	228 ± 26	38,515 ± 422
	3×10^4	158 ± 17	16,783 ± 398
	1×10^4	164 ± 32	8728 ± 927
Non-adherent fraction	3×10^5	178 ± 14	1467 ± 54
	1×10^5	43 ± 34	119 ± 72
	3×10^4	56 ± 19	48 ± 29
	1×10^4	59 ± 7	26 ± 15
EBV-B	3×10^5	2710 ± 187	6305 ± 342
	1×10^5	2352 ± 18	4376 ± 212
	3×10^4	713 ± 47	1308 ± 334
	1×10^4	359 ± 95	478 ± 71

* TLC TAB 31 (2×10^4 cells/well) were cultured for 40 hr with 2700 rads irradiated PBMC, adherent or non-adherent PBMC or 3300 rads irradiated EBV-B in the absence and presence of nickel sulphate (10^{-4} M).

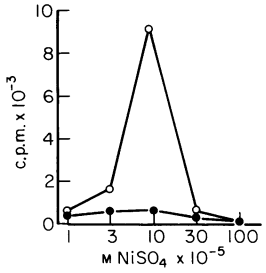

Figure 1. The comparison of the capacity of 1×10^5 peripheral blood cells (○) and 2×10^4 EBV-B cells (●) as APC in the proliferative response of TLC TAB 31 to a varying nickel sulphate concentration.

B cell non-responder TAB 31 did proliferate to either of the B-cell fractions (Table 3). These data indicate that EBV-B cells are acceptable as functional representations of the normal B-cell population.

Role of sialic acid

Differences in cell surface glycosylation levels have proved to restrict antigen presentation by resting B cells (Frohman & Cowing). We have eliminated sialic acid by neuraminidase treatment to detect whether sialic acid restricts antigen presentation by activated B cells to the TLC. As shown in Table 4, decreased levels of cell surface sialic acid did not result in the capacity of the EBV-B cells to present antigen to TLC TAB 31.

Table 3. Comparison of the antigen-presenting capacities of freshly isolated B cells and EBV-B

APC	No. APC	Proliferative response (c.p.m. ± SD)*	
		No nickel	Nickel
TLC TAB 31			
PBMC	3×10^5	179 ± 28	5620 ± 1952
EBV-B	4×10^4	1648 ± 52	1675 ± 273
B cells	2×10^5	34 ± 1	264 ± 110
	1×10^5	30 ± 11	228 ± 214
TLC TAB 19			
PBMC	3×10^5	74 ± 21	8635 ± 324
EBV-B	4×10^4	984 ± 5	7309 ± 2831
B cells	2×10^5	78 ± 17	7193 ± 1298
	1×10^5	84 ± 9	5921 ± 436

* TLC (2×10^4 cells per well) were cultured for 40 hr with 2700 rads irradiated PBMC, 3300 rads irradiated EBV-B or 1000 rads irradiated freshly isolated B cells in the presence or absence of 10^{-4} M nickel sulphate.

Table 4. Attempts to restore the response of TLC TAB 31 to nickel presented by B cells by the treatment of EBV-B with neuraminidase or cytokines

APC	Pretreatment†	Proliferative response* (c.p.m. ± SD)	
		No antigen	Antigen
Exp. 1			
PBMC	None	42 ± 20	13,616 ± 2452
EBV-B	None	345 ± 31	352 ± 21
EBV-B	Neuraminidase	575 ± 101	804 ± 51
Exp. 2			
PBMC	None	310 ± 34	13,926 ± 65
EBV-B	None	1795 ± 156	1741 ± 11
EBV-B	Neuraminidase	1527 ± 83	1578 ± 10
EBV-B	IL-1	1784 ± 156	1861 ± 67
EBV-B	IL-6	1550 ± 179	1761 ± 120
EBV-B	IFN-γ	1380 ± 16	1319 ± 71
EBV-B	IL-4	1616 ± 105	1365 ± 26

* See Table 3.

† EBV-B were pretreated with 100 U/ml neuraminidase for 30 min or with 1000 U/ml rIL-1β 5000 U/ml rIL-6, 1000 U/ml rIFN-γ or 200 U/ml IL-4 for 4 days.

Role of HLA-II molecules

The class II MHC restrictions of antigen recognition of the TLC were examined in order to search for a relation between B-cell responsiveness and certain restriction determinants. Using blocking antibodies against HLA-II subloci and panels of differently tissue-typed peripheral blood APC, it was found that

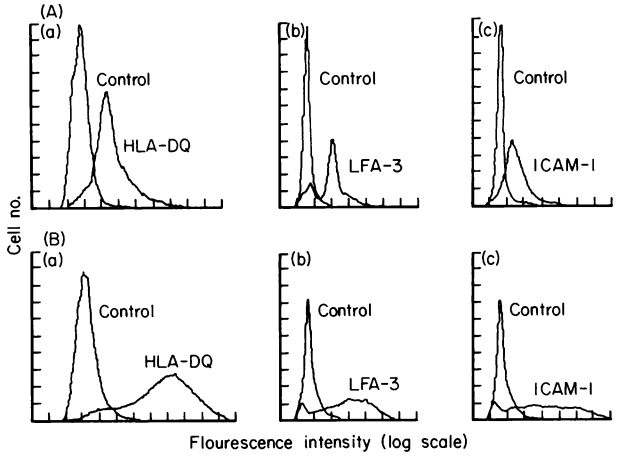

Figure 2. The comparison of expressions of HLA-DQ (a), LFA-3 (b) and ICAM-1 (c) by the monocyte fraction of peripheral blood (A) and by EBV-B Donor 2. (B). Controls are cells incubated with the fluorescent second layer only.

the TLC from Donor 1 were all restricted by HLA-DR1 or HLA-DR3, whereas there was no relation between HLA restriction and responsiveness to antigen presented by B-cells (Table 1). In contrast, B-cell non-responder TLC from Donor 2 all appeared to be restricted by HLA-DQ. All these HLA-DQ-restricted TLC were differently allo-restricted, indicating that these clones were not cloned from a single ancestor T cell. This observation stressed the notion that unresponsiveness to antigen presented by B cells from Donor 2 may result from an insufficient HLA-DQ expression by these B cells. Cytofluorimetric analysis of HLA-DQ expression, however, showed that EBV-B from Donor 2 expressed even higher amounts of HLA-DQ than autologous monocytes (Fig. 2).

Role of adhesion molecules

Pieces of evidence suggest that antigen presentation is restricted by adhesion molecules that bridge APC and T cells, since antibodies against these structures block antigen recognition by proliferating T cells (Davignon *et al.*, 1980; Dougherty *et al.*, 1988; Bierer *et al.*, 1988). Our recent studies have demonstrated the simultaneous requirement of formation of two bridges that are formed by ICAM-1 and LFA-3 on APC interacting with LFA-1 and CD2 on T cells, respectively. Therefore, it may be postulated that defective antigen presentation by activated B cells results from an absent or poor expression of ICAM1 or LFA-3. However, cytofluorimetric analysis clearly showed that EBV-B express more ICAM1 or LFA-3 than monocytes (Fig. 2).

Role of soluble factors

Soluble factors like IL-1β, IFN-γ, IL-4 and IL-6 are known to raise the state of activation of several cell types. Most of these factors activate APC, resulting in an enhancement of their antigen-presenting capacities (Inaba & Steinman 1984; Zlotnik *et al.*, 1987; Helle *et al.*, 1988; Hawrylowicz & Unanue, 1988). However, EBV-B that have been pretreated with recombinant

Table 5. EBV-B cells fail to induce both IL-2 receptor expression and IL-2 production by TLC TAB 31

APC	IL-2R expression*	IL-2 production† (U/ml)
Monocytes	++	3·3
EBV-B	±	—

* IL-2 receptor (IL-2R) expression was determined microscopically after 40 hr of culture. ++ means high expression, ± means weak expression.

† IL-2 production was measured after 24 hr of culture in a bioassay using a IL-2-dependent CTL cell line.

Table 6. Attempts to explain the failure of antigen-presenting B cells by the failure of production of soluble factors

APC	Addition	Proliferative response (c.p.m. ± SD)* No nickel	Nickel
Exp. 1			
PBMC	—	310 ± 34	13,926 ± 65
EBV-B	—	1795 ± 156	1741 ± 11
	rIL-1 (1000 U/ml)	1299 ± 32	1189 ± 120
	rIL-6 (5000 U/ml)	1286 ± 12	1534 ± 144
	rIFN-γ (1000 U/ml)	1123 ± 45	1163 ± 150
	rIL-2 (100 U/ml)	1717 ± 177	1813 ± 186
	rIL-4 (200 U/ml)	1200 ± 4	1347 ± 233
Exp. 2			
PBMC	—	72 ± 53	7354 ± 179
	Culture supernatant†	3020 ± 470	9678 ± 285
EBV-B	—	506 ± 3	589 ± 127
	Culture supernatant	986 ± 26	1910 ± 1035

* See Table 3.

† Culture supernatant of 4×10^4 TAB 31 activated by nickel presented by 4×10^5 PBMC for 24 hr.

versions of these proteins were still unable to present antigen to TLC TAB 31 (Table 4).

T-cell activation requires the induction of expression of a receptor for growth factor IL-2R and the induction of production of IL-2 (Palacios, 1982). The failure of responsiveness to antigen presented by B cells may result from a selective failure of one of these events. Although the antigen-specific activation of TAB 31 by monocytes is accompanied by both the induction of IL-2 production and IL-2R expression, culture of TAB 31 in the presence of EBV-B and antigen did not result in any of these events (Table 5). As may be expected from the low IL-2R expression, the addition of rIL-2 to these cultures did not induce antigen-specific proliferation with B-cell APC of TAB 31 (Table 6). Recently, it was demonstrated that the T cell product IL-4 can also act as a T-cell growth factor (Spits *et al.*, 1987).

Addition of rIL-4 could not restore proliferation of TAB 31 to B-cell APC (Table 6).

IL-1 produced by APC is required as a secondary signal in the activation of T cells (Scala & Oppenheim, 1983). Yet, addition of recombinant IL-1β (rIL-1β) could not reverse unresponsiveness to B cells of TLC TAB 31 (Table 6). If the lack of any soluble factor caused unresponsiveness of TAB 31, this factor should be present in the supernatants of TAB 31 stimulated by antigen presented by monocytes. Using these supernatants still no proliferation of the TAB 31 was induced by antigen presented by B-cell APC (Table 6).

DISCUSSION

We have studied APC heterogeneity by an analysis of the ability of panels of TLC to respond to diverse antigens presented by either B cells or (adherent) APC from peripheral blood. All these TLC specifically responded to antigen presented by peripheral blood APC, but only a limited number of these TLC were able to respond to these antigens when presented by B cells as APC.

The differential antigen-presenting function is probably not due to differential antigen processing, since B cells and macrophages are equally capable of processing soluble proteins and consequently antigen-specific activation of antigen-primed bulk T cells (Shimonkevitz *et al.*, 1983). The exact molecular mechanisms of antigen processing are not fully understood and possible differences in processing by APC subsets cannot be entirely ruled out. Therefore, it cannot be excluded that B-cell APC present different antigen epitopes, resulting in differential reactivities of cloned T-cells. This seems to be unlikely in the case of nickel ions, since there are strong indications that nickel ions bind directly to the cell surface and do not require processing (Kapsenberg *et al.*, 1988a, unpublished experiments).

A number of other possibilities was considered to explain the limited capacity of B cells to present antigen. Firstly, we examined a possible relation with the expression of cell surface structures that are involved in antigen presentation. Antigen recognition can be restricted by cell surface structures that are masked by sialic acid. Neuraminidase treatment could enhance recognition of HLA molecules on resting B cells by uncloned T cells and restore the responsiveness of allospecific TLC (Cowing & Chapdelaine, 1983). Moreover, Fohrman & Cowing (1988) have demonstrated that sialic acid restricts antigen presentation by resting B lymphocytes. In addition, we found a similar restricting role for sialic acid in antigen presentation by peripheral blood APC to TLC that respond well to antigen presented by Langerhans' cells (Kapsenberg *et al.*, 1988b). The present observations indicate that sialic acid residues do not play a critical role in the defective antigen-presenting capacity of B cells.

The level of HLA-II expression seemed not to be restrictive in B-cell antigen presentation, since B cells showed a much higher expression of HLA-II molecules than monocytes. The possibility should be considered that HLA-II molecules are expressed differently on B cells and monocytes. According to the number of genes (2α and 2β genes), cells might have the potential to express several HLA-DQ heterodimers. Indeed, cell lines have been shown to express two or more HLA-DQ molecules (Karr *et al.*, 1984; Johnson & Wank, 1984). A point of consideration, therefore, is the possibility that B cells might have an expression of a subtype of HLA-DQ, which some autologous TLC can, but other autologous TLC cannot, recognize. Yet no data are available indicating a different subtype expression of HLA-DQ molecules on different cell types of the same individual.

As mentioned above, antigen presentation is dependent on the exposure of functional epitopes of the cell adhesion molecules ICAM1 and LFA-3. Thus far, there are no reports on functional APC heterogeneity that is caused by differential expression of these molecules. Our quantitative analysis of expression of functional epitopes of these molecules revealed higher expressions of EBV-B compared to monocytes. These results seem to rule out the obvious possibility that the failure of antigen-presenting B cells is caused by a low expression of these molecules.

Secondly, the role of soluble factors was examined. T-cell proliferation requires the presence of IL-1 (Scala & Oppenheim, 1983). Addition of IL-1β to B-cell APC non-responder TLC did not restore the response. Nor did other growth factors, such as IL-2, IL-4, IL-6 and soluble factors produced by stimulated TLC.

Matsui *et al.* (1987) have described a human Ig-specific murine TLC that responded well to Ig presented by spleen cells, but that failed to respond to Ig presented by B-cell APC. The supernatant of this clone, stimulated with antigen and spleen APC, resulted in a modest proliferative response of the TLC to antigen presented by B cells. This enhancement probably reflects the enhancing effect of cytokines (IL-4) on the antigen-presenting capacities of resting B cells, as reported earlier. The present data do not favour a role for any known soluble factor or its receptors in the failure of antigen presentation by activated B cells.

A likely explanation, therefore, could be that the failing response is caused by the lack of a signal that is mediated by direct cell contact through contact sites different from HLA-II-TcR, CD2-LFA-3 and LFA-1-ICAM1. For instance, in addition to activation structures like the TcR-CD3 complex, CD2 and LFA-1, T-cell proliferation can be induced via CD28 (Hara & Hansen, 1985) and Tp103 (Fleischer, 1987). Their physiological counter structures are unknown, but may be present on APC. It may be hypothesized that B-cell non-responder TLC require activation via CD28 or Tp103 and that the counter structures are absent on B cells.

The present investigation was focused mainly on the analysis of structures on the cell surface of the B cells and no obvious abnormality was found. Further analysis of the expression of structures on the cell surface of T cells that are critical in antigen presentation may provide new clues for the elucidation of the failure of B cells to present antigen to the majority of T cells.

ACKNOWLEDGMENTS

We wish to thank Drs J. Aten and L. de Waal for the assistance with cytofluorimetric analysis and tissue typing, respectively.

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