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mAb 104, a new monoclonal antibody, recognizes the B7 antigen that is expressed on activated B cells and HTLV-1-transformed T cells

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SUMMARY

A new monoclonal antibody (mAb) of the IgG1 subclass, mAb 104, has been obtained after immunization of mice with the Burkitt lymphoma cell line Jijoye. It only weakly binds to a small proportion of non-activated normal B cells and binds to a larger proportion of *in vitro*-activated normal B cells. All tested Epstein–Barr virus (EBV)-transformed B-cell lines, Burkitt lymphoma cell lines and freshly isolated follicular B-lymphoma cell preparations strongly bound mAb 104. mAb 104 did not bind to peripheral monocytes or tested myelomonocytic cell lines, or to resting and activated normal T cells, T-cell lines and T-cell clones. However, the recognized antigen is expressed on HTLV-1-infected T-cell lines and HTLV-1-transformed T-cell clones. mAb 104 immunoprecipitates, from Jijoye cell lysates, a single polypeptide with an apparent MW of 45,000–60,000 and an isoelectric point of 5·6. Competition studies with the anti-B7 antibody (Freedman *et al.*, 1987) demonstrated that mAb 104 and the anti-B7 block each others' binding. Furthermore, mAb 104 binds to transfected COS cells (Freeman *et al.*, 1989) expressing the B7 antigen. Thus mAb 104 and anti-B7 define the same antigen. The restricted distribution of the 104/B7 antigen to activated B cells and HTLV-1-transformed T cells may make it a useful marker for the study of pathological states linked to lymphocyte activation and for the functional study of B-cell subpopulations.

INTRODUCTION

Several monoclonal antibodies (mAb) have been developed that are specific for antigens expressed on activated lymphocytes (Knapp *et al.*, 1989). The best studied activation antigens appearing on B lymphocytes following activation, such as the 4F2 antigen, the transferrin receptor (CD71), or the interleukin-2 (IL-2)-binding protein (CD25/Tac), are not specific for either T- or B-lymphocyte lineages. Several mAb have been reported that detect activation antigens, such as $Fc\varepsilon R_2$/CD23 (Bonnefoy *et al.*, 1987), Ba (Kikutani *et al.*, 1986), Bac-1 (Suzuki *et al.*, 1986), B5 (Freedman *et al.*, 1985) and B7 (Freedman *et al.*, 1987), on B cells. The expression of these antigens was found to be induced by polyclonal B-cell activators such as anti-IgM antibodies or phorbol esters. Recently, $Fc\varepsilon R_2$/CD23 expression was found to be strongly up-regulated by IL-4 on normal B cells (Defrance *et al.*, 1987), whereas Ba (CDw78) expression was up-regulated by conditioned medium from phytohaemagglutinin (PHA)-activated T cells (Kikutani *et al.*, 1986).

With the long-term goal of establishing monoclonal antibodies specific for cytokine receptors, and the assumption that activated B cells may express more cytokine receptors, mice were immunized with the Burkitt lymphoma cell line Jijoye. These cells were chosen because they respond to IL-4 with an increased $Fc\varepsilon R_2$/CD23 expression (Rousset *et al.*, 1988). In this study, the development and characterization of a new monoclonal antibody, mAb 104, that recognizes the recently identified B-cell activation antigen B7, is reported. Furthermore, it is demonstrated that this antigen is expressed on HTLV-1-transformed T cells and freshly isolated lymphoma B cells.

Abbreviations: EBV, Epstein–Barr virus; $Fc\varepsilon R_2$/CD23, low-affinity Fc receptor for IgE of lymphocytes; HTLV-1, human T-lymphotropic virus 1; K_d, dissociation constant; mAb, monoclonal antibody; SDS-PAGE, sodium dodecylsulphate-polyacrylamide gel electrophoresis.

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MATERIALS AND METHODS

Monoclonal antibody production and reagents

mAb 104 (IgG1) was produced, according to standard protocols (Bonnefoy *et al.*, 1987), by immunizing mice (Iffa Ciedo, Les Oncins, France) with Jijoye cells (a Burkitt lymphoma cell line). mAb were isolated from ascitic fluid by high-pressure liquid chromatography (HPLC). *Staphylococcus aureus* Cowan I (SAC) and ionomycin were from Hoechst-Calbiochem (La Jolla, CA). Anti-IgM antibody coupled to beads (Immuno-

beads) was from BioRad (Richmond, CA). Phorbol myristate acetate (PMA) and phorbol dibutyrate (PDB) were from Sigma (St Louis, MO). Fluorescein isothiocyanate (FITC)-conjugated $F(ab')_2$ fragments of goat anti-mouse Ig were from Grub (Vienna, Austria). The anti-B5 (Freedman *et al.*, 1985), anti-B7 (Freedman *et al.*, 1987) and the anti-CD14 (Mo2) (Todd, Nadler & Schlossman, 1981) mAb have been described elsewhere and are of the IgM isotype.

Cell sources and culture media

Most of the cell lines were obtained from the American Type Culture Collection (Rockville, MD). The Epstein–Barr virus (EBV)-transformed lymphoblastoid lines UD53, 61, 68 were produced at UNICET by Dr F. Rousset. The Burkitt lymphoma cell lines BL2, 30 and 70 were kindly provided by Dr G. Lenoir (International Agency for Research on Cancer, Lyon, France). These cell lines were cultured in complete medium: RPMI-1640 supplemented by 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine (all from Flow Laboratories, Irvine, Ayrshire, U.K.). The HTLV-1-infected T-cell lines 8166 and C91PL were kindly provided by Dr L. Gazzolo (Faculté de Médecine Alexis Carrel, Lyon, France). The IL-2-dependent T-cell clones ($CD4^+$) and the HTLV-1-infected T-cell clones were produced at UNICET (Yssel *et al.*, 1989). Mononuclear cell suspensions were obtained from tonsils, spleens, peripheral blood, cord blood or bone marrow aspirates, as described previously (Bonnefoy *et al.*, 1987). To obtain purified B-cell populations, T cells were removed from the mononuclear cells by rosetting twice, as described by Defrance *et al.* (1987). For activation assays, purified B cells were cultured in complete medium as described above.

Flow cytometry analysis and immunoprecipitation analysis

Fluorescence staining was performed in microtrays as described earlier (Bonnefoy *et al.*, 1987), and staining was analysed with a FACS 440 (Becton-Dickinson, Sunnyvale, CA). Immunoprecipitation studies from Jijoye cells were performed with mAb 104 as described previously (Bonnefoy *et al.*, 1987). Cell-surface labelling and immunoprecipitation from COS cells transfected with a cDNA encoding for the B7 antigen were performed according to Freeman *et al.* (1989).

RESULTS

Reactivity of mAb 104 with various cell populations and cell lines

The hybridoma clone 104 which produces the monoclonal antibody mAb 104 was generated by fusion of the NS1 myeloma cells with spleen cells obtained from a mouse immunized with Jijoye cells (a Burkitt lymphoma cell line). mAb 104 is of the IgG1 isotype. The reactivity of mAb 104 with various mononuclear cell populations and various cell lines was analysed by flow cytometry (Table 1). mAb 104 did not significantly bind to mononuclear cells obtained from peripheral blood, cord blood or from bone marrow (not shown). It very weakly bound to some tonsil and spleen mononuclear cell and purified B-cell suspensions (Fig. 1a). Blood mononuclear cells activated with PHA did not significantly bind mAb 104. Stimulation of purified tonsil B cells with anti-IgM antibody (Fig. 1b) coupled to beads (5 μg/ml) or SAC (0·05%) or PMA (10 ng/ml) or a combination of PDB (1 ng/ml) and ionomycin (1 μg/ml) resulted in induction of the antigen recognized by mAb 104 (Ag 104) on a proportion of the cells. A mean of 15% of the cells was found to express this antigen after 3 days of activation with anti-IgM antibody or PMA. The PDB/ionomycin combination was the most potent inducer of Ag 104, since 35% of tonsil B cells were positive after only 24 hr of activation. mAb 104 bound to cells from three out of five tested chronic lymphoid B leukaemias, five out of five tested follicular B lymphomas but none of three tested acute myeloid leukaemias (not shown).

Table 1. Reactivity* of mAb 104 with various mononuclear cell preparations and cell lines

	% positive cells	
	CD20	mAb 104
Blood MNC (6)†	10±4	3±2
5 days PHA blasts (6)‡	8±4	4±2
Tonsil MNC (4)	56±4	5±4
Tonsil B cells (6)	98±2	9±5
Anti-IgM-activated B cells (10)‡	94±4	15±5
SAC-activated B cells (8)‡	ND	26±9
PMA-activated B cells (3)‡	ND	12±6
PDB + ionomycin-activated B cells (4)‡	ND	35±10
Spleen MNC (3)	42±5	4±3
Spleen B cells (3)	97±2	11±3
T-cell lines and IL-2-dependent T-cell clones (Molt 4, Jurkat, CEM, PEER), (827, 837, 714, Geo2)		<1
HTLV-1-infected T-cell lines and HTLV-1-transformed T-cell clones (HUT 102, 8166, C91PL), (827p19, 837p19, B21p19)		100
Myelomonocytic, erythroid and nul cell lines (HL60, U937, KG1), (K562), (Reh 6)		<1
Burkitt lymphoma and EBV-transformed lymphoblastoid cell lines (Jijoye, Daudi, Raji, BL2, BL30, BL70) (RPMI 8866, JY, UD53, UD61, UD68)		100

* Determined by indirect immunofluorescence and flow cytometry.

† Number of analysed samples.

‡ Peripheral blood mononuclear cells (10^6/ml) were activated with 1 μg/ml PHA (Welcome, Dartford, Kent, U.K.). Highly purified tonsil B cells were activated for 72 hr with insolubilized anti-IgM antibody (5 μg/ml) or SAC (0·05%) or PMA (10 ng/ml), or activated for 24 hr with PDB (1 ng/ml) plus ionomycin (1 μg/ml).

ND, not determined.

In order to examine further the specificity of mAb 104, a large panel of cell lines was examined for Ag 104 expression by flow cytometry (Table 1). mAb 104 was unreactive with T-cell lines and myelomonocytic cell lines. It did not react with IL-2-dependent T-cell clones. It bound very weakly to the plasmacytoma cell line RPMI-8226 and did not bind to the null cell line Reh 6 and the myeloma cell line U266 (not shown). It reacted strongly with all the assayed EBV-transformed lymphoblastoid cell lines. Ag 104 was also expressed on Burkitt lymphoma cell lines, but the level of expression was lower than that observed on EBV-transformed lymphoblastoid cell lines, except for Jijoye cells (Fig. 1c), which strongly expressed Ag 104. 122,000 binding sites could be detected on Jijoye cells by equilibrium binding analysis (not shown) with iodinated mAb 104 (K_d: 1·5 nM).

Interestingly, all tested HTLV-1-infected T-cell lines (HUT 102, 8166, C91PL) and HTLV-1-transformed T-cell clones

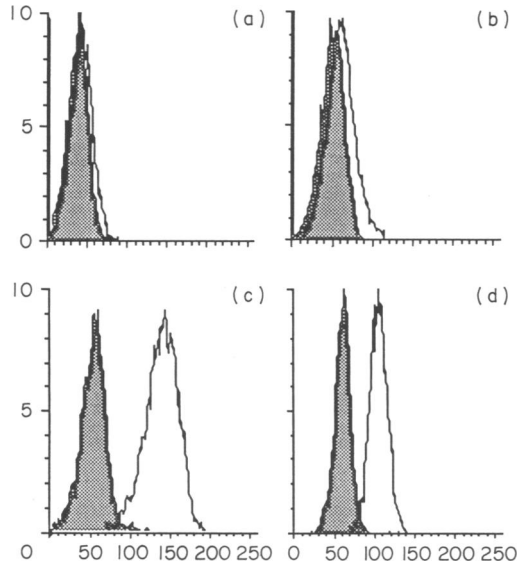

Figure 1. Binding of mAb 104 to various cell populations and cell lines. (a) Tonsil B cells; (b) anti-IgM antibody-activated tonsil B cells (5 μg/ml, 72 hr); (c) Jijoye cells (Burkitt lymphoma cell line); and (d) HTLV-1-transformed T-cell clone 827 p19. Dark histograms represent background staining with a non-related control mAb (anti-denatured human interferon-gamma). Abscissa, log fluorescence intensity; ordinate, relative cell number.

(827p19, 837p19, B21p19) expressed Ag 104 (Fig. 1d). These clones did not express the B-cell antigens CD20, CD22 or CD23, but one of them (837p19) slightly expressed CD21 (not shown). Ag 104 was found to be expressed on HTLV-1-infected T-cell clones at similar levels during both the IL-2-dependent and IL-2-independent phases described by Yssel *et al.* (1989). HUT 102 cells expressed 26,000 mAb 104 binding sites (not shown). Taken together the data indicate that mAb 104 binds to an antigen expressed on activated B cells and HTLV-1-transformed T cells.

mAb 104 recognizes the B7 antigen

Preliminary experiments demonstrated that the epitope recognized by mAb 104 is sensitive to pronase treatment. Thus Jijoye cells were surface labelled with ^{125}I-Na and lactoperoxidase. Labelled cell lysates were immunoprecipitated with mAb 104 and the precipitate was analysed by SDS–PAGE and autoradiography. Data in Fig. 2 show that mAb 104 is able to precipitate a 45,000–60,000 MW polypeptide under both reducing (Fig. 2a) and non-reducing conditions (not shown), with an isoelectric point of 5·6 (Fig. 2b). From RPMI-8866 cell lysates, mAb 104 precipitated a larger component of 55,000–70,000 MW (not shown).

The binding pattern of mAb 104 to various mononuclear cell suspensions or cell lines and the molecular weight of the recognized antigen led to the hypothesis that mAb 104 and the anti-B7 antibody (Freedman *et al.*, 1987) recognized the same antigen. Thus competition studies were performed. The anti-B7 antibody totally blocks the binding of biotinylated mAb 104 (and vice versa; not shown). This inhibition was specific since anti-HLA-DR (IgG1) or anti-B5 (IgM) did not inhibit the binding of mAb 104 to Jijoye cells (not shown). Thus, mAb 104

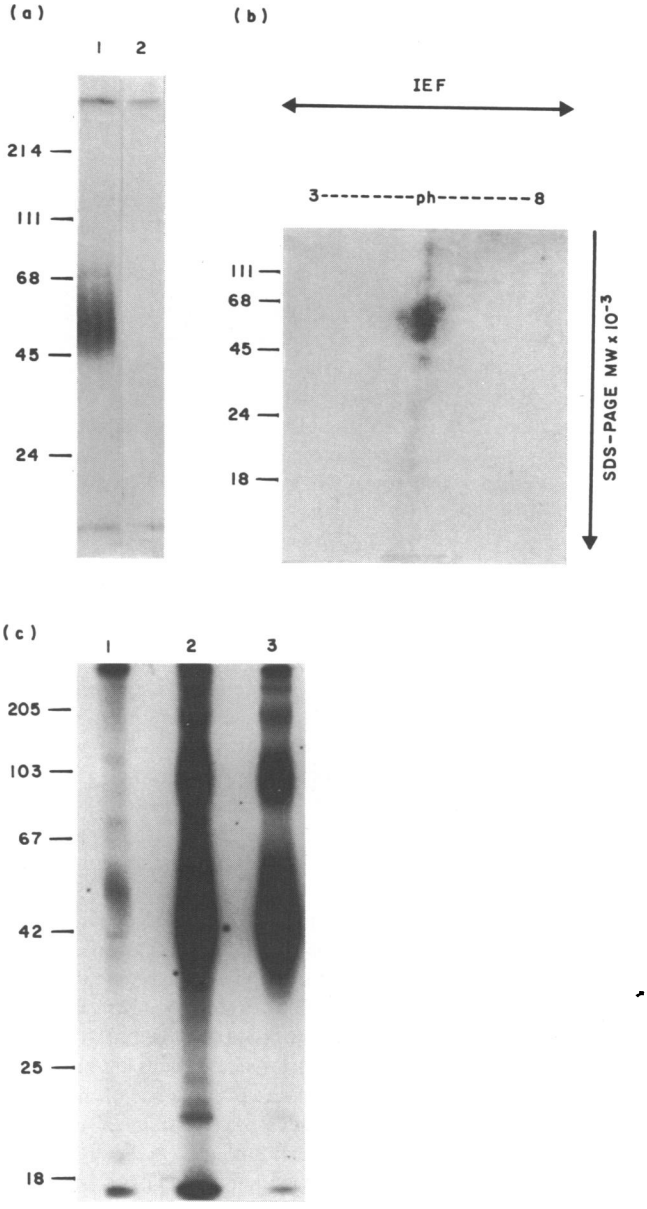

Figure 2. Electrophoretic analysis of the antigen immunoprecipitated with mAb 104. Jijoye cells (a, b) or B7-transfected COS cells (c) were enzymatically labelled with ^{125}I-Na and lysed. After preclearing, the Jijoye cell lysates were incubated with mAb 104 (a1) or with an IgG1 mAb control (a2) coupled to Sepharose beads. The two-dimensional electrophoresis (b) was performed as described by Bonnefoy *et al.* (1987). (c) B7-transfected COS cell lysates were immunoprecipitated as follows: (c1) anti-CD14, (c2) anti-B7, (c3) mAb 104. Immunoprecipitated proteins were analysed by 12·5% (a, b) or 10% (c) SDS–PAGE and autoradiography. Numbers on the left lane of each panel represent the MW $\times 10^{-3}$ of coelectrophoresed prestained MW standards.

recognizes an epitope on the B7 antigen, which is identical to or close to the epitope recognized by the anti-B7 mAb. The identities of the B7 and 104 antigens were further examined by immunoprecipitation. As seen in Fig. 2c, a 48,000 MW protein was specifically immunoprecipitated from B7-transfected COS cells by the anti-B7 mAb (lane 2) but not by anti-CD14 (lane 1). The mAb 104 immunoprecipitated a polypeptide of identical

molecular weight from B7-transfected COS cells (lane 3). Interestingly, a component of 90,000–100,000 MW was also specifically precipitated by both antibodies.

DISCUSSION

In the present study, a new IgG1 monoclonal antibody has been characterized; mAb 104, which binds to activated B cells. Competition studies and immunoprecipitation studies demonstrated that it recognizes the B-cell activation antigen B7, defined by the IgM antibody anti-B7 (Freedman *et al.*, 1987). This antigen is virtually not expressed on resting B cells and monocytes, not at all on normal T cells but is expressed on activated B cells. As described earlier, this antigen is strongly expressed on EBV-transformed B-cell lines and Burkitt lymphoma cell lines. Of importance is the finding that mAb 104 strongly bound to five out of five freshly isolated B lymphomas and three out of five tested chronic lymphocytic B leukaemias.

Whereas mAb 104 did not react with resting T cells, activated normal T cells, various T-cell lines or IL-2-dependent cell clones, it strongly bound to all tested HTLV-1-infected T-cell lines and HTLV-1-transformed T-cell clones. The Ag 104/B7 was detected during the IL-2-dependent and IL-2-independent phases following the HTLV-1 infection of the T-cell clones (Yssel *et al.*, 1989). Whether the expression of Ag 104/B7 by HTLV-1-transformed T cells has any relationship with the transformation phenomenon requires further studies. HTLV-1-transformed T cells have also been described as expressing CD23/FεR$_2$ (Nutman *et al.*, 1987), an antigen earlier considered as specific of activated B cells but now recognized as more widely distributed (Gordon *et al.*, 1989). However, the HTLV-1-infected clones studied herein did not display significant CD23 expression (data not shown). CD21, another B-cell specific antigen that has been described as being expressed on the HTLV-1-infected T-cell line HUT 102 (Schultz *et al.*, 1986), was also found to be expressed on one out of the three tested HTLV-1-infected T-cell clones. HTLV-1-transfected cells are known to express high levels of Tac/CD25 as a consequence of induction of trancription factors that binds to kB enhancer elements present in the CD25 gene (Greene, Böhnlein & Ballard, 1989). It will be of interest to establish whether the HTLV-1-induced expression of Ag 104/B7 involves similar or different mechanisms.

Ag 104/B7, expressed on Jijoye cells, is a protein of 45,000–60,000 MW and pI 5·6. However, some heterogeneity in size was found since mAb 104 precipitated a 55,000–70,000 MW protein from RPMI-8866 cells. It is not known at present whether the heterogeneity is at the glycosylation or the protein level. In addition to the 45,000–60,000 MW protein, mAb 104 and anti-B7 precipitated a 90,000–100,000 MW protein from B7-transfected COS cells. Interestingly, Western blot analysis performed on partially purified Ag 104/B7 (obtained from Jijoye cell lysate passed over immobilized mAb 104) also revealed the presence of a 90,000–100,000 MW component (data not shown). This 90,000–100,000 MW protein might represent a non-dissociable dimer of the 45,000–60,000 MW Ag 104/B7. Such dimers have been described for other B-cell antigens, such as CD40 (Braesch-Andersen *et al.*, 1989), but their role remains to be formally established. In this context, the dimeric form of the epidermal growth factor (EGF) receptor seems to be the active form of the receptor which transduces the mitogenic signal of EGF (Schlessinger, 1988). Before similar conclusions may be drawn for Ag 104/B7, one will have to establish its function. The structure and expression of Ag 104/B7 is certainly compatible with its being a receptor for a growth or differentiation factor (Freeman *et al.*, 1989), although it is probably not a component of the IL-4 receptor since mAb 104 does not inhibit the binding of ^{125}I-IL-4 (not shown). The functional study of Ag 104/B7-positive cells and the cytokine-induced Ag 104/B7 regulation, presently under investigation, may provide important clues for the understanding of the role of this antigen.

The restricted distribution of Ag 104/B7 to activated B cells and HTLV-1-transformed T cells may make it a useful marker for the study of pathological states linked to lymphocyte activation, and mAb 104 is certainly worth considering for *in vivo* or *ex vivo* eradication of leukaemic cells.

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REFERENCES

Bonnefoy J.Y., Aubry J.P., Peronne C., Wijdenes J. & Banchereau J. (1987) Production and characterization of a monoclonal antibody specific for the human lymphocyte low affinity receptor for IgE: CD23 is a low affinity receptor. *J. Immunol.* **138,** 2970.

Braesch-Andersen S., Paulie S., Koho H., Nika H., Aspenström P. & Perlmann P. (1989) Biochemical characteristics and partial amino acid sequence of the receptor-like human B cell and carcinoma antigen CDw40. *J. Immunol.* **142,** 562.

Defrance T., Aubry J.P., Rousset F., Vanbervliet B., Bonnefoy J.Y., Arai N., Takebe Y., Yokota T., Lee F., Arai K., de Vries J.E. & Banchereau J. (1987) Human recombinant interleukin 4 induces Fcε receptors (CD23) on normal human B lymphocytes. *J. exp. Med.* **165,** 1459.

Freedman A.S., Boyd A.W., Anderson K.C., Fisher D.C., Schlossman S.F. & Nadler L.M. (1985) B5, a new B cell-restricted activation antigen. *J. Immunol.* **134,** 2228.

Freedman A.S., Freeman G., Horowitz J.C., Daley J. & Nadler L.M. (1987) B7, a B cell-restricted antigen that identifies preactivated B cells. *J. Immunol.* **139,** 3260.

Freeman G.J., Freedman A.S., Segil J.M., Lee G., Withman J.F. & Nadler L.M. (1989) B7, a new member of the Ig superfamily with unique expression on activated and neoplastic B cells. *J. Immunol.* **43,** 2714.

Gordon J., Flores-Romo L., Cairns J.A., Millsum M.J., Lane P.J., Johnson G.D. & MacLennan I.C.M. (1989) CD23: a multifunctional receptor/lymphokine? *Immunol. Today*, **10,** 153.

Greene W.C., Böhnlein E. & Ballard D.W. (1989) HIV-1, HTLV-1 and normal T-cell growth: transcriptional strategies and surprises. *Immunol. Today*, **10,** 272.

Kikutani H., Kimura R., Nakamura H., Sato R., Muraguchi A.,

KAWAMURA N., HARDY R.R. & KISHIMOTO T. (1986) Expression and function of an early activation marker restricted to human B cells. *J. Immunol.* **136,** 4019.

KNAPP W., RIEBER P., DÖRKEN B., SCHMIDT R.E., STEIN H. & BORNE A.E.G.KR.V.D. (1989) Towards a better definition of human leukocyte surface molecules. *Immunol. Today*, **10,** 253.

NUTMAN T.B., DELESPESSE G., SARFATI M. & VOLKMAN D.J. (1987) T cell-derived IgE-binding factors. *J. Immunol.* **139,** 4049.

ROUSSET F., DE WAAL MALEFIJT R., SLIERENDREGT B., AUBRY J.P., BONNEFOY J.Y., DEFRANCE T., BANCHEREAU J. & DE VRIES J.E. (1988) Regulation of Fc receptor for IgE (CD23) and class II MHC antigen expression on Burkitt's lymphoma cell lines by human IL-4 and IFN-γ. *J. Immunol.* **140,** 2625.

SCHLESSINGER J. (1988) Signal transduction by allosteric receptor oligomerization. *TIBS*, **13,** 443.

SCHULTZ T.F., PETZER A., STRAUDER R., EIGENTLER A. & DIERICH M.P. (1986) Expression of CR2 (C3d/EBV-receptor) on HTLV-1 transformed human T-cells. *Abstracts of the 6th International Congress Immunology*, Toronto 2.51.14, p. 188.

SUZUKI T., SANDERS S.K., BUTLER J.L., GARTLAND G.L., KOMIYAMA K. & COOPER M.D. (1986) Identification of an early activation antigen (Bac-1) on human B cells. *J. Immunol.* **137,** 1208.

TODD R.F., NADLER L.M. & SCHLOSSMAN S.F. (1981) Antigens on human monocytes identified by monoclonal antibodies. *J. Immunol.* **126,** 1435.

YSSEL H., DE WAAL MALEFYT R., DUC DODON M., BLANCHARD D., GAZZOLO L., DE VRIES J.E. & SPITS H. (1989) Human T cell Leukemia/Lymphoma virus Type I infection of a $CD4^+$ proliferative/cytotoxic T cell clone progresses in at least two distinct phases based on changes in function and phenotype of the infected cells. *J. Immunol.* **142,** 2279.