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Expression and modulation of cell surface determinants on human adult and neonatal monocytes

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SUMMARY

The difference between newborn and adult mononuclear cells in the antigen dose required for optimal antibody production *in vitro* can be ascribed to differences in the antigen-presenting capacities of the respective monocytes (Van Tol *et al.*, 1984b). We have therefore studied the expression of cell surface determinants on human neonatal and adult monocytes by the use of monoclonal antibodies to membrane proteins including MHC antigens. No difference was observed in the expression of LeuM3 with regard to both the percentage of positive cells and the density of the respective determinant. In contrast, neonatal cells express the antigens OKM5, LFA1, OKM1 and LeuM5 at a lower density than adult cells do. The same holds for β_2-microglobulin, but neonatal and adult monocytes express MHC class I α-chains at a similar density, whereas among the class II MHC antigens, HLA-DR is significantly more highly expressed on neonatal cells. This difference remains after treatment *in vitro* with γ-interferon (γ-IFN). Treatment with γ-IFN also resulted in a less dense expression of the LeuM3 antigen. Preincubation of monocytes with LeuM3 monoclonal antibody partially abrogates subsequent upregulation of class II MHC antigens by γ-IFN, a phenomenon observed with both neonatal and adult monocytes. These data indicate a functional involvement of LeuM3 with the cellular action of γ-IFN. Taken together, the cell surface phenotype of neonatal monocytes is that of a highly efficient antigen presenting cell.

Keywords neonatal monocytes class I MHC antigens class II MHC antigens antigen presentation human monocyte determinants

INTRODUCTION

Morphological and functional development of the immune system in a number of mammalian species is not fully established at the time of birth. In man, only a limited capacity to mount a specific antibody response after antigenic stimulation *in vivo* does exist. Although IgM-type antibodies against protein antigens may be synthesized at birth, IgG-type antibody formation is delayed and polysaccharide antibody formation starts only at the end of the second year of life (Pabst & Kreth, 1980).

The ability to mount a specific antibody response against protein antigens is dependent not only on antigen-reactive B lymphocytes but also on accessory cells presenting antigen in concert with MHC class II antigens to T helper/inducer cells. In previous studies we have shown that stimulation *in vitro* of human cord blood mononuclear cells (CBMC) with a variety of T cell dependent antigens like ovalbumin, *Helix pomatia* haemocyanin, tetanus toxoid, etc. results in the generation of antigen-specific plaque forming cells (Van Tol *et al.*, 1983; 1984a). Analysis of the cellular requirements of the antibody response induced *in vitro* disclosed the participation of antigen-presenting monocytes, B lymphocytes and immunoregulatory T cells including T helper/inducer, T suppressor/inducer and T suppressor/effector cells. An intriguing observation appeared to be that, while the magnitude of the plaque-forming cell response of CBMC was similar to that found in adult peripheral blood mononuclear cells (PBMC), the antigen dose required to induce an optimal response consistently was 100-fold lower in the case of CBMC as compared to PBMC, irrespective of the antigen used (Van Tol *et al.*, 1983; 1984a). In subsequent studies this difference in antigen dose appeared to be determined primarily by the neonatal monocyte and preliminary studies suggesting that differences in antigen handling and processing capacity do exist between neonatal and adult monocytes (Van Tol *et al.*, 1984b).

In order to explore these differences in more detail we investigated the expression of a number of monocyte membrane

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determinants by use of monoclonal antibodies. The most notable difference was that neonatal monocytes express class II MHC products in higher densities than adult monocytes, a difference maintained following upregulation by treatment *in vitro* with γ-interferon.

MATERIALS AND METHODS

Cell preparation

PBMC from healthy adult volunteers and CBMC from healthy neonates whose mothers went through an uncomplicated full-term pregnancy, were obtained by centrifugation of heparinized blood over Ficoll-Isopaque gradients ($\delta = 1{\cdot}077$ g/cm^3; Pharmacia, Uppsala, Sweden). For some experiments, monocytes were isolated by plastic adherence on culture flasks (Costar, Cambridge, MA) for 1 h at 37°C in minimal essential medium (MEM) supplemented with 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml).

Stimulation of cells

PBMC and CBMC were stimulated with formyl-methyl-leucyl-phenylalanine (FMLP; Sigma, St Louis, MO, USA) or ionomycin (Calbiochem, La Jolla, CA, USA) after the method of Springer *et al.* (1984). Briefly, cells were stimulated for 15 min at 37°C at a concentration of 10×10^6 cells/ml in RPMI-1640 medium (Gibco, Buffalo, NY) supplemented with 10% human AB-serum, penicillin, streptomycin and 2 mM L-glutamine in 4 ml culture tubes (Falcon, Oxnard, CA). Dose response studies showed 1 μM to be optimal concentration for the use of FMLP and ionomycin. Stimulation with recombinant γ-interferon (γ-IFN; Biogen, Geneva, Switzerland) was performed on plastic adherent cells from PBMC or CBMC by incubating these for 20 h at 37°C at a density of 1×10^6/ml in supplemented RPMI-1640 in the presence or absence of γ-IFN.

Membrane staining of mononuclear cells

In order to detect expression of cell surface antigens $0{\cdot}5 \times 10^6$ cells were suspended in 10 μl of appropriately diluted monoclonal antibody (see below) in MEM, 1% (w/v) bovine serum albumin (BSA), 0·05% (w/v) Na-azide, and incubated for 30 min at 4°C under constant shaking. Cells were then washed three times with MEM-BSA-azide and stained with a goat-anti-mouse secondary antibody conjugated to FITC (Becton Dickinson, Mountain View, CA). Cells were then washed again and subsequently counterstained with anti-LeuM3 monoclonal antibody conjugated to phycoerythrin (see below). Before analysis, cells were resuspended in sheath fluid.

Monoclonal antibodies

Characterization of neonatal and adult monocyte surface determinants was carried out using a panel of monoclonal antibodies. Designation and specificities of these reagents are listed in Table 1.

Flowcytometry

Cells were subjected to two-colour flowcytometric analysis using a FACS Analyzer (Becton Dickinson) equipped with a Ushio (Tokyo, Japan) model 102 D Xenon lamp DF 485/22 excitation and DF530/30 (green) and LP 570 (red) emission filters. Data were analysed using Consort 30 software (Becton Dickinson). The expression of a given cell surface antigen is represented as the mean fluorescence intensity (MFI; arbitrary units). Results from several experiments are expressed as the mean MFI ± the standard error of the mean (s.e.m.). From data for adults and neonates which were obtained on the same date, ratios of MFI were calculated according to the formula: ratio = MFI neonatal cells/MFI adult cells. Results from several paired analyses are represented as the mean ratio of MFI (MRFI) ± s.e.m.

Statistical analysis

A rank order test was used to determine if MRFIs differed significantly from 1. $P < 0{\cdot}05$ was considered significant.

Table 1. Specificity and source of monoclonal antibodies used in this study

Monoclonal antibody	Molecular weight (kD)	Predominant reactivity	Clone	Reference	Source
LeuM3 (CD14)	(53)	Monocytes	Mϕ-P9	Dimitriou-Bona *et al.* (1983)	1
OKM5	88	Monocytes	OKM5	Shen *et al.* (1983)	2
LeuM2	120	Monocytes	Mac120	Raff *et al.* (1980)	1
LFA1 (CD11a)	180/95	Lymphocytes, monocytes, granulocytes	CLB-LFA-1/1	Sanchez-Madrid *et al.* (1983)	3
OKM1 (CD11b)	160/95	Monocytes, granulocytes	OKM1	Sanchez-Madrid *et al.* (1983)	2
LeuM5 (CD11c)	150/95	Monocytes	S-HCL-3	Sanchez-Madrid *et al.* (1983)	1
HLA-A,B,C	44	Class I pos. cells	B9.12.1	Rebaï & Malissen (1983)	4
β_2-microglobulin	12	Class I pos. cells	B11.G6	Rebaï & Malissen (1983)	4
HLA-DP	34	B cells, monocytes	B7/21	Watson *et al.* (1983)	4
HLA-DQ	34	B cells, monocytes	SPV-L3	Spits *et al.* (1984)	4
HLA-DR	36	B cells, monocytes, activated T cells	L243	Lampson & Levy (1980)	1

1, Becton Dickinson, Mountain View, CA, USA
2, Ortho, Raritan, NJ, USA.
3, Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.
4, Gift from Dr F. Koning, Leiden, The Netherlands.

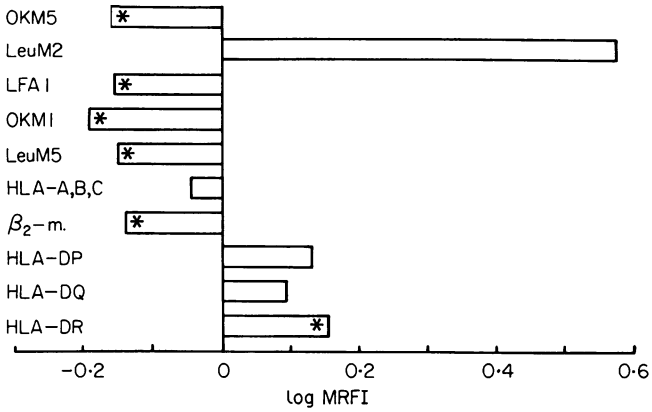

Fig. 1. Expression of cell surface antigens on adult and neonatal LeuM3-positive mononuclear cells. Results are shown as the mean ratio of MFI (MRFI). A log MFRI >0 indicates a higher density of a given cell surface antigen on neonatal monocytes and a log MFRI <0 indicates a higher density on adult monocytes. The s.e.m. of the MRFI varied between 7·1 and 64·8%, with a mean of 16·8% $\pm$ 5·4%. All MRFI are based on five determinations, except the one for HLA-DR, which is based on eight. * MRFI differing significantly from 1 ($P<0·05$).

RESULTS

Expression of cell surface antigens

Expression of cell surface antigens on cord blood and peripheral blood monocytes was studied by using a panel of monoclonal antibodies (Table 1). As neonatal mononuclear cell suspensions may contain granulocytes and sometimes also erythroblasts, FACS analysis solely on the basis of volume and side scatter signals does not provide a reliable basis for comparison of adult and neonatal cell surface antigen expression.

However, the LeuM3 antigen, which has been recognized as a pan-monocytic marker by Dimitriou-Bona *et al.* (1983), is expressed at similar densities both on adult and neonatal monocytes (mean MFI: 132·1 $\pm$ 28·5 ($n=7$) vs 104 $\pm$ 18·1 ($n=12$), respectively). The ratio of expression of LeuM3 between paired samples of neonatal and adult monocytes ($n=8$) did not differ significantly. For this reason expression of all other antigens studied in this paper is represented as the MFI of green fluorescence within the compartment of LeuM3-positive (i.e. strongly red) fluorescing cells (Fig. 1).

OKM5 and LeuM2 are monocyte-specific markers which were included in this study because subsets have been delineated which show distinct antigen-presenting capabilities (Raff, Picker & Stobo, 1980; Shen *et al.*, 1983). OKM5 was expressed to a significantly greater density on adult than on neonatal monocytes. In contrast LeuM2 was expressed to a greater density on neonatal monocytes, but this difference was not significant (Fig. 1).

In addition cell adhesion antigens presumably involved in cellular interactions between, e.g. monocytes and T cells, and detectable by anti-LFA1, anti-OKM1, and anti-LeuM5 (Sanchez-Madrid *et al.*, 1983), were included in this study. Adult monocytes appeared to express LFA1, OKM1 and LeuM5 at a higher MFI than neonatal cells did.

Expression of MHC antigens

Expression of MHC class I antigens on neonatal and adult LeuM3-positive cells was tested with monoclonal antibodies directed against a monomorphic determinant on HLA-A, HLA-B and HLA-C α-chains and a monoclonal antibody against β_2-microglobulin (Table 1). Expression of β_2-microglobulin was significantly higher on adult than on neonatal cells, while the MRFI for the class I α-chain antibody showed no significant deviation from 1 in these cases (Fig. 1).

MHC class II expression was studied using monoclonal antibodies against monomorphic determinants on the respective HLA-DP, HLA-DQ and HLA-DR α-chains. The expression of the HLA-DP, HLA-DQ, and HLA-DR antigens was higher on neonatal cells, although the differences are significant for DR only (Fig. 1).

Stimulation with FMLP and ionomycin

As mentioned before, we noted significant differences between adult and neonatal monocytes as to the expression of cellular

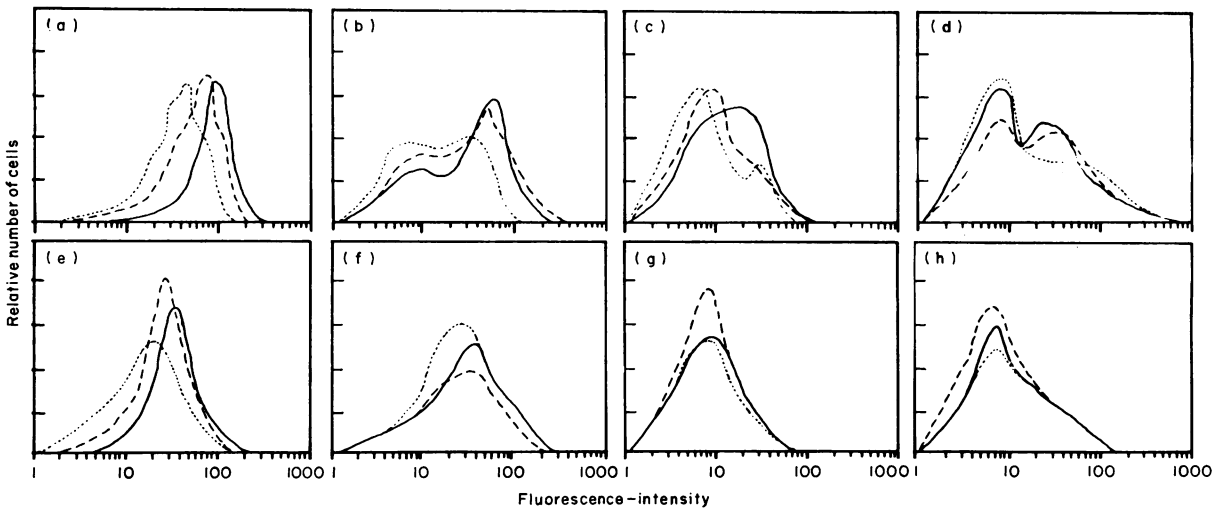

Fig. 2. Effect of FMLP and ionomycin on expression of cell surface antigens. (a–d) PBMC and (e–h) CBMC were incubated with (———) FMLP, (– – –) ionomycin, or (. . .) medium for 15 min at 37°C. Expression of (a,e) LFA1, (b,f) OKM1, (c,g) LeuM5, and (d,h) HLA-DR on LeuM3-positive cells is shown. One representative experiment out of three is shown.

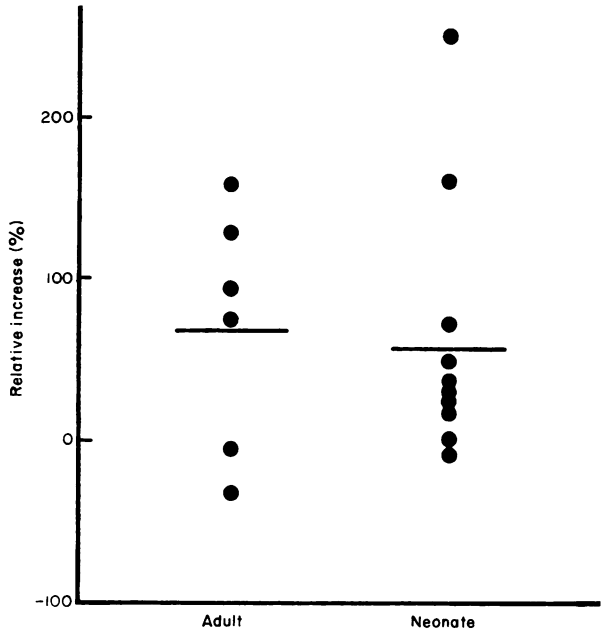

Fig. 3. Effect of γ-IFN on expression of HLA-DR on adherent cells. Plastic adherent cells isolated from PBMC and CBMC were incubated with 300 U/ml γ-IFN for 20 h at 37°C. Relative increase (%) of HLA-DR expression on LeuM3-positive cells incubated with γ-IFN as compared to LeuM3-positive cells incubated without γ-IFN is shown. The bar indicates mean increase. Each data point reflects an individual experiment.

adhesion molecules (Fig. 1). Springer *et al.* (1984) have described how the expression of these molecules on granulocytes could be augmented by using the chemotactic factor FMLP or a calcium-ionophore. Therefore we studied the effect of FMLP and the calcium-ionophore ionomycin (Liu & Hermann, 1978) on the MFI of LFA1, OKM1, LeuM5, and (as a control) HLA-DR on adult and neonatal monocytes. As shown in Fig. 2, stimulation of adult and neonatal cells resulted in equal enhancement of LFA1 for CBMC and PBMC. OKM1 was enhanced for PBMC but hardly for CBMC, while there was a slight increase of LeuM5 expression on PBMC but not on CBMC upon stimulation with FMLP. The expression of HLA-DR did not change on both cell types.

Stimulation of adherent cells with γ-IFN

As it is well documented that γ-IFN enhances expression of class II MHC antigens, we studied the stimulation of plastic adherent cells from PBMC and CBMC by this lymphokine. Overnight incubation resulted in increased expression of HLA-DR both on neonatal and adult cells; relative to adherent cells not incubated with γ-IFN an increase of $59 \pm 26\%$ ($n = 10$) vs $69 \pm 31\%$ ($n = 6$) was found for adult and cord cells respectively (Fig. 3). The extent of increase did not differ between neonatal and adult adherent cells.

Besides this increase in expression of HLA-DR we observed a concomitant decrease in the expression of the LeuM3 antigen. This decrease involved both the percentage of LeuM3-positive cells and the MFI of the antigen on the positive fraction (Fig. 4). Although this observation was made both for adult and

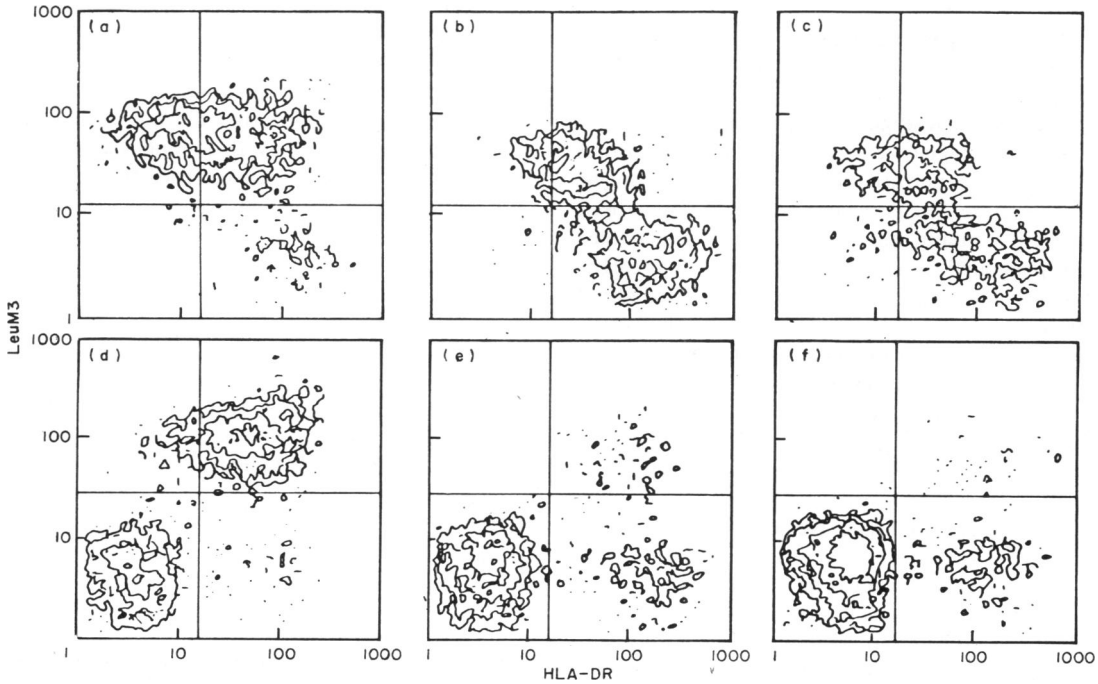

Fig. 4. Decreased expression of LeuM3 caused by γ-IFN. Adherent mononuclear cells were incubated with (a,d) 0, (e) 250, (b) 300, or (c,f) 1000 U/ml γ-IFN for 20 h at 37°C. Expression of HLA-DR and LeuM3 on adherent (a–c) PBMC and (d-f) CBMC is shown. Proportion of LeuM3-positive cells: (a) 85%; (b) 50%; (c) 44%; (d) 51%; (e) 8%; (f) 5%. One representative experiment out of six is shown.

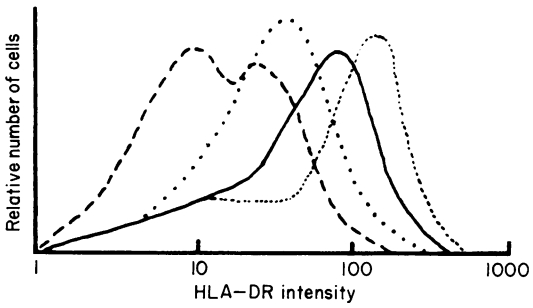

Fig. 5. Effect of preincubation with anti-LeuM3 before stimulation with γ-IFN. Adherent PBMC were incubated with (- - -,———) anti-LeuM3 or (. . ,. . .) medium for 30 min at 4°C and subsequently with 300 U/ml (———,. . .) γ-IFN or (- - -, . . .) medium for 20 h at 37°C. The expression of HLA-DR on LeuM3-positive cells is shown. One representative experiment out of five is shown.

neonatal adherent cells, the decrease of LeuM3 expression was more pronounced on the latter.

In order to establish the possible relation between the LeuM3 antigen and the action of γ-IFN, we incubated adult adherent cells with anti-LeuM3 before they were stimulated with γ-IFN. Pretreatment of adherent cells with anti-LeuM3 caused γ-IFN to increase the MFI of HLA-DR expression to a lesser extent than without preincubation. In a tyical experiment (Fig. 5), γ-IFN caused an increase of HLA-DR intensity by a factor 3·5 without anti-LeuM3 pretreatment, while this was reduced to a factor 2·3 after incubation with anti-LeuM3. Pretreatment with another monocyte-specific monoclonal antibody (anti-OKM1) did not have such an effect (data not shown).

DISCUSSION

In this paper we have presented the phenotypical analysis of neonatal and adult human monocytes. Monocytes can be physically separated from mononuclear cell suspensions by a variety of techniques such as differential centrifugation, plastic adherence or particle phagocytosis. The latter two procedures can lead to membrane perturbation and hence monocyte activation. Furthermore, the enriched monocyte populations thus obtained still contain, e.g. plastic adherent activated lymphocytes or phagocytic granulocytes. A further bias can be introduced because the complicating factors mentioned above do not equally apply to neonatal and adult cells (Prindull *et al.*, 1975). For these reasons we have chosen to perform phenotypical analysis in two-colour immunofluorescence of Ficoll-isolated mononuclear cell suspensions in which expression of a given determinant is related to the expression of the pan-monocytic antigen LeuM3 (CD14; Dimitriou-Bona *et al.*, 1983). A premise for this approach is that LeuM3 behaves as a pan-monocytic marker for neonatal cells. We believe this to be true since in functional and phenotypical studies we have never observed monocyte-related reactivities in LeuM3-negative populations or lymphocyte reactivities within LeuM3-positive cells. Formally it cannot be excluded that a minor subpopulation of neonatal monocytes exists which does not express LeuM3.

Two markers, recognized by the monoclonal antibodies OKM5 and LeuM2, have been included in this study as they were originally reported to be present on subpopulations of monocytes which were efficient antigen-presenting cells (Raff *et al.*, 1980; Shen *et al.*, 1983). However, the staining pattern of OKM5 and (in accordance with Gonwa & Stobo, 1984) LeuM2 does not reveal separate subpopulations, since all monocytes express these determinants. Only the expression of OKM5 appeared to differ significantly on neonatal and adult monocytes.

Molecules of the so-called Leu-CAM family function as accessory molecules by facilitating cellular interactions (see e.g. Fischer *et al.*, 1986). Their importance is dramatically illustrated in the clinical condition resulting from a deficiency of these molecules (leucocyte adhesion deficiency; Anderson & Springer, 1987). Significant differences were observed in the cellular expression of these accessory molecules on neonatal and adult monocytes. The failure of FMLP and ionomycin in upregulating LeuM5 expression is in accordance with results which have been previously published by Springer, Miller & Anderson (1986).

Whether or not a given cell can function as an antigen-presenting cell is determined by its expression of class II MHC products. The direct involvement of class II molecules follows from the finding that monocytes from class II MHC deficient patients fail to function as antigen-presenting cells (Zegers *et al.*, 1986). Recently, the physical interaction between antigenic peptides and class II molecules has been described (Buus *et al.*, 1987).

When tested with a panel of class II specific monoclonal antibodies neonatal monocytes are found to express class II MHC products at a higher density than adult monocytes do. The difference is significant for HLA-DR only, although expression of HLA-DP and HLA-DQ seems also higher on neonatal monocytes. This result is at variance with those previously reported by several other authors (e.g. Zlabinger, Mannhalter & Eibl, 1983; Stiehm *et al.*, 1984; Durandy, Brami & Griscelli, 1985). However, these authors made use of conventional immunofluorescence microscopy, an approach which allows only a semi-quantitative determination of fluorescence intensities. Besides, they did not identify monocytes by means of counterstaining with a monocyte-specific monoclonal antibody. The difference between class II expression of neonatal and adult monocytes remains following treatment *in vitro* with γ-IFN (Fig. 3). It is however unlikely that the high density of class II molecules on neonatal monocytes results from exposure *in vivo* to γ-IFN, since neonatal T lymphocytes have been demonstrated to be poor γ-IFN producers (Wakasugi & Virelizier, 1985).

Besides the recognized effect of γ-IFN on class II expression, this lymphokine also has been demonstrated to modulate a variety of other monocyte cell surface determinants (Esparza, Fox & Schreiber, 1986; Mentzer, Faller & Burakoff, 1986). An unexpected finding was the apparent loss of LeuM3 following γ-IFN treatment, an observation recently also made by others (Firestein & Zvaifler, 1987; Rambaldi *et al.*, 1987). Down regulation of CD14 is not invariably linked with increased class II expression since treatment with LPS results in enhanced DR expression, but leaves the expression of CD14 unaltered (Todd *et al.*, 1985). In a number of systems it has been demonstrated that upon ligand-receptor interaction the newly formed complex disappears from the cell surface (through internalization, shedding or otherwise). To address the question whether CD14 would serve as the γ-IFN receptor on monocytes we have

preincubated monocytes with anti-LeuM3. Thus pretreated monocytes showed a less marked increase in class II expression following exposure to γ-IFN as compared to non-pretreated monocytes. Monocytes pretreated with another monoclonal antibody of the same subclass (anti-OKM1, IgG2b) did not have any effect on the action of γ-IFN. CD14 cannot be the universal γ-IFN receptor since its expression is restricted to cells of the monocyte/macrophage lineage. Recent data however suggest that the γ-IFN receptor on monocytes differs from non-haematopoietic cells (Orchansky, Rubinstein & Fischer, 1986). From limited structural data, a molecular weight of 100–125×10^3 kD for γ-IFN receptors can be derived (Sarkar & Gupta, 1984; Littman, Faltynek & Baglioni, 1985) while CD14 has a molecular weight of 53 kD. It is therefore unlikely that anti-LeuM3 would react with the γ-IFN receptor itself but with a molecule which could function as an associated structure as in the case of the CD3/T cell receptor complex or the Tac/interleukin 2 receptor complex (Hatakeyama, Minamoto & Taniguchi, 1986).

We have previously demonstrated that neonatal monocytes enable optimal antibody production *in vitro* at a 100-fold lower antigen dose as compared to adult peripheral monocytes (Van Tol *et al.*, 1984a,b). In this paper we have shown that the key-structures involved in the process of antigen presentation (class II MHC products) are expressed at a higher density on neonatal than on adult monocytes. The phenotype of a neonatal monocyte is therefore compatible with that of a highly efficient antigen-presenting cell. The functional consequences of the particular phenotype of neonatal monocytes are currently being addressed in quantitative studies on antigen processing and presentation.

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REFERENCES

ANDERSON, D.C. & SPRINGER, T.A. (1987) Leukocyte adhesion deficiency: an inherited deficiency in the Mac-1, LFA-1, and p150,95 glycoproteins. *Ann. Rev. Med.* **38,** 175.

BUUS, S., SETTE, A., COLON, S.M., MILES, C. & GREY, H.M. (1987) The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. *Science* **235,** 1353.

DIMITRIOU-BONA, A., BURMESTER, G.R., WATERS, S.J. & WINCHESTER, R.J. (1983) Human mononuclear phagocyte differentiation antigens. I. Patterns of antigenic expression on the surface of human monocytes and macrophages defined by monoclonal antibodies. *J. Immunol.* **130,** 145.

DURANDY, A., BRAMI, C. & GRISCELLI, C. (1985) The effects of indomethacin administration during pregnancy on women's and newborns' T-suppressor lymphocyte activity and on HLA class II expression by newborns' leukocytes. *Am. J. Reprod. Immunol. Microbiol.* **8,** 94.

ESPARZA, I., FOX, R.I. & SCHREIBER, R.D. (1986) Interferon-γ-dependent modulation of C3b receptors (CR1) on human peripheral blood monocytes. *J. Immunol.* **136,** 1360.

FIRESTEIN, G.S. & ZVAIFLER, N.J. (1987) Down regulation of human monocyte differentiation antigens by interferon γ. *Cell. Immunol.* **104,** 343.

FISCHER, A., DURANDY, C., STERKERS, G. & GRISCELLI, C. (1986) Role of the LFA-1 molecule in cellular interactions required for antibody production in humans. *J. Immunol.* **136,** 3198.

GONWA, T.A. & STOBO, J.D. (1984) Differential expression of Ia molecules by human monocytes. *J. Clin. Invest.* **74,** 859.

HATEKEYAMA, M., MINAMOTO, S. & TANIGUCHI, T. (1986) Intracytoplasmic phosphorylation sites of Tac antigen (p55) are not essential for the conformation, function, and regulation of the human interleukin 2 receptor. *Proc. natn Acad. Sci. USA* **83,** 9650.

LAMPSON, L.A. & LEVY, R. (1980) Two populations of Ia-like molecules on a human B cell line. *J. Immunol.* **125,** 393.

LITTMAN, S.J., FALTYNEK, C.R. & BAGLIONI, C. (1985) Binding of recombinant ^{125}I-interferon to receptors on human cells. *J. biol. Chem.* **260,** 1191.

LIU, C. & HERMANN, T.E. (1978) Characterization of ionomycin as a calcium ionophore. *J. biol. Chem.* **253,** 5892.

MENTZER, S.J., FALLER, D.V. & BURAKOFF, S.J. (1986) Interferon-induction of LFA-1-mediated homotypic adhesion of human monocytes. *J. Immunol.* **137,** 108.

ORCHANSKY, P., RUBINSTEIN, M. & FISCHER, D.G. (1986) The interferon-receptor on human monocytes is different from the one on nonhematopoietic cells. *J. Immunol.* **136,** 169.

PABST, H.F. & KRETH, H.W. (1980) Ontogeny of the immune response as a basis of childhood disease. *J. Pediatrics* **97,** 519.

PRINDULL, G., PRINDULL, B., PALTI, Z. & YOFFEY, J.M. (1975) Phagocytic cells in cord blood. *Biol. Neonate* **27,** 318.

RAFF, H.V., PICKER, L.J. & STOBO, J.D. (1980) Macrophage heterogeneity in man. A subpopulation of HLA-DR bearing macrophages required for antigen-induced T cell activation also contains stimulators for autologous-reactive T cells. *J. exp. Med.* **152,** 581.

RAMBALDI, A., YOUNG, D.C., HERMANN, F., CANNISTRA, S.A. & GRIFFIN, J.D. (1987) Interferon-γ induces expression of the interleukin 2 receptor gene in human monocytes. *Eur. J. Immunol.* **17,** 153.

REBAÏ, N. & MALISSEN, B. (1983) Structural and genetic analysis of HLA class I molecules using monoclonal antibodies. *Tissue Antigens* **2,** 107.

SANCHEZ-MADRID, F., NAGY, J.A., ROBBINS, E., SIMON, P. & SPRINGER, T.A. (1983) A human leukocyte differentiation antigen family with distinct α-subunits and a common β subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. *J. exp. Med.* **158,** 1785.

SARKAR, F.H. & GUPTA, S.L. (1984) Receptors for human γ-interferon: binding and crosslinking of ^{125}I-labeled recombinant human γ-interferon to receptors on WISH cells. *Proc. natn Acad. Sci. USA* **81,** 560.

SHEN, H.H., TALLE, M.A., GOLDSTEIN, G. & CHESS, L. (1983) Functional subsets of human monocytes defined by monoclonal antibodies: a distinct subset of monocytes contains the cells capable of inducing the autologous mixed lymphocyte culture. *J. Immunol.* **130,** 698.

SPITS, H., BORST, J., GIPHART, M.J., COLIGAN, J., TERHORST, C. & DE VRIES, J.E. (1984) HLA-DC antigens can serve as recognition elements for human cytotoxic T lymphocytes. *Eur. J. Immunol.* **14,** 299.

SPRINGER, T.A., THOMPSON, W.S., MILLER, L.J., SCHMALSTIEG, F.C. & ANDERSON, D.C. (1984) Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. *J. exp. Med.* **160,** 1901.

SPRINGER, T.A., MILLER, L.J. & ANDERSON, D.C. (1986) p150,95, the

third member of the Mac-1, LFA-1 human leukocyte adhesion glycoprotein family. *J. Immunol.* **136,** 240.

STIEHM, E.R., SZTEIN, M.B., STEEG, P.S., MANN, D., NEWLAND, C., BLAESE, R.M. & OPPENHEIM, J.J. (1984) Deficient DR antigen expression on human cord blood monocytes: reversal with lymphokines. *Clin. Immunol. Immunopathol.* **30,** 430.

TODD, R.F., ALVAREZ, P.A., BROTT, D.A. & LIN, D.Y. (1985) Bacterial lipopolysaccharide, phorbol myristate acetate, and muramyl dipeptide stimulate the expression of a human monocyte surface antigen, Mo3e. *J. Immunol.* **135,** 3869.

VAN TOL, M.J.D., ZIJLSTRA, J., HEIJNEN, C.J., KUIS, W., ZEGERS, B.J.M. & BALLIEUX, R.E. (1983) Antigen-specific plaque-forming cell response of human cord blood lymphocytes after *in vitro* stimulation by T cell-dependent antigens. *Eur. J. Immunol.* **13,** 390.

VAN TOL, M.J.D., ZIJLSTRA, J., ZEGERS, B.J.M. & BALLIEUX. R.E. (1984a) Antigen-induced plaque-forming cell responses in cultures of peripheral blood mononuclear cells of human neonates and infants. *J. Pediatrics* **105,** 738.

VAN TOL, M.J.D., ZIJLSTRA, J., THOMAS, C.M.G., ZEGERS, B.J.M. & BALLIEUX, R.E. (1984b) Distinct role of neonatal and adult monocytes in the regulation of the *in vitro* antigen-induced plaque-forming cell response in man. *J. Immunol.* **134,** 1902.

WAKASUGI, N. & VIRELIZIER, J.-L. (1985) Defective interferon-γ production in the human neonate. *J. Immunol.* **134,** 167.

WATSON, A.J., DE MARS, R., TROWBRIDGE, I.S. & BACH, F.H. (1983) Detection of a novel human class II HLA antigen. *Nature* **304,** 358.

ZEGERS, B.J.M., RIJKERS, G.T., ROORD, J.J., KONING, F., SCHUURMAN, H.J., HEIJNEN, C.J., KUIS, W., VAN DE BERG, H. & STOOP, J.W. (1986) Defective expression of MHC-antigens on mononuclear cells in two siblings with combined immunodeficiency: delineation of the class II antigen defect. In: *Progress in Immunodeficiency Research and Therapy* Vol. II (eds. J. Vossen & C. Griscelli) p. 97. Excerpta Medica, Amsterdam.

ZLABINGER, G.J., MANNHALTER, J.W. & EIBL, M.M. (1983) Cord blood macrophages present bacterial antigen (*Escherichia coli*) to paternal T cells. *Clin. Immunol. Immunopathol.* **28,** 405.