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Effect of IFN-γ on Expression of HLA in Bare-Lymphocyte Syndrome-Like Cell Line HAJ

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Abstract. We compared HLA antigen expression on new B-lymphoblastoid cell line (B-LCL) HAJ with that on B-LCLs expressing normal HLA levels as well as on B-LCLs derived from bare lymphocyte syndrome (BLS) patients and *in vitro* mutated B-LCLs of BLS-like phenotype. HAJ cells had no expression of HLA class II and low expression of class I antigens similarly to some of BLS B-LCLs, although HAJ cell line was derived from lymphocytes of HLA class I- and class II-normally expressing donor. HAJ cells displayed B lymphocyte markers, surface immunoglobulin and CD19. Culture of HAJ cells in the presence of interferon γ resulted in HLA class I antigen upregulation, but did not restore class II expression. The cell line HAJ may prove useful for studies on factors influencing HLA class I cell surface expression.

Key words: HLA expression defect, B-lymphoblastoid cell line, flow cytometry, interferon γ.

Introduction

Bare lymphocyte syndrome (BLS) is a rare severe immunodeficiency disease manifesting with a lack of human leukocyte antigen (HLA) class II molecules although the class II genes are present. As these molecules play a pivotal role in antigen presentation, BLS patients are prone to bacterial, fungal and protozoan infections and die in early childhood unless successfully transplanted with normal allogeneic bone marrow[6, 20]. Numerous B-lymphoblastoid cell lines (B-LCLs) were derived from BLS patients by immortalisation with Epstein-Barr virus (EBV). These cell lines provide information on mechanisms controlling the expression of HLA class II genes. At least four, and possibly more, complementation groups of cell lines with different genetic defects are already known (Table 1). In addition, several *in vitro* mutated cell lines exhibiting similar or other defects were also described[6, 20]. We have recently reported on a new B-LCL, HAJ. It was found to have BLS-like phenotype (i.e. no class II expression) although it was derived from a renal insufficiency patient with normal class II expression[14]. A comparison of HLA class I and class II expression on HAJ cells with that on cell lines belonging to four complementation groups is presented here.

Interferon γ (IFN-γ) is potent upregulator of major histocompatibility complex (MHC) class I and class II gene expression[7–9, 22, 23]. Since we have found that HAJ cells do not express class II antigens (ref. [12, 14] and this

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Table 1. Cell lines used in this study

Cell line	Genetic defect
PAJ	None known (derived from a healthy donor); normal phenotype
FG	Mutation in phosphomannomutase gene; derived from a type I carbohydrate-deficient glycoprotein syndrome patient; normal HLA expression
HAJ	Nature of the defect not definitely known (CIITA? See Discussion); low cell surface expression of HLA class I antigens, no expression of HLA class II genes; derived from renal insufficiency patient with normal HLA expression
Daudi	Mutations in both alleles of β_2-microglobulin resulting in lack of cell surface of HLA class I antigens, but normal expression of HLA class II antigens; derived from Burkitt lymphoma cells
721.174	*In vitro* immunoselected deletion of entire HLA class II region including TAP genes from one chromosome and of entire HLA complex from the other chromosome; no HLA class II expression and extremely low cell surface expression of HLA class I antigens due to the lack of TAP
T2	721.174 × CEM cell hybrid lacking both HLA-bearing chromosomes of CEM partner; HLA phenotype identical to that of 721.174 parental line
RJ2.2.5	*In vitro* induced mutation of CIITA gene in cell line Raji resulting in lack of HLA class II gene expression; complementation group A
BLS-1	Mutation in transcription factor RFX-B (RFX-ANK) gene; derived from a BLS patient; phenotype as above; complementation group B
SJO	Mutation of transcription factor RFX5 gene; derived from a BLS patient; phenotype as above; complementation group C
6.1.6	*In vitro* induced mutation of transcription factor RFXAP gene; phenotype as above; complementation group D

report) and display only low levels of cell surface class I antigens (see Results), we examined the effect of IFN-γ on class I and class II antigen expression on these cells.

Materials and Methods

Cell lines. Characteristics of cell lines used in this work are presented in Table 1.

HAJ and PAJ B-LCLs were described elsewhere[11, 14, 15]. PAJ cells express HLA-DR, -DQ and -DP class II as well as HLA class I antigens, and served here as a positive control. FG cell line was obtained from Dr. Gert Matthijs, Katholieke Univerzitet Leuven, Belgium, due to courtesy of Dr. Bogna Litwińska, State Institute of Hygiene, Warsaw, Poland. It expresses normal levels of HLA class I and class II antigens (NOWAK et al., manuscript in preparation) and therefore also served here as a positive control. Daudi[4] cell line was generously offered by Dr. Antonio Juretić, Department of Research, Kantonsspital Basel, Switzerland. Daudi cells served here as HLA class I-negative, class II-positive control.

Following cell lines were used here as HLA class II-negative cells: non-transfected SJO cell line[2] was a kind gift of Dr. Janice S. Blum, University of Indiana, Indianapolis, USA. Five cell lines were obtained from Dr. Susan Kovats, University of Washington, Seattle, USA, with kind help from Dr. Stefan Martin from the same University and Dr. Stephanie Weinreich, Dutch Red Cross Blood Transfusion Centre, Amsterdam, the Netherlands: three cell lines, BLS-1[10], SJO, and 6.1.6[5] were obtained as HLA-DRA and hygromycin-resistance double transfectants, BLS-1 (DRA hygro), SJO (DRA hygro), and 6.1.6 (DRA hygro), that did not express cell surface HLA-DR antigens because DRB gene did not function in these cells due to defects of transcription factors (Dr. S. Kovats, personal communication; see also Table 1 and Fig. 2). T2[19] hygromycin-resistant cell line and nontransfected RJ2.2.5[1] cell line were also obtained from Dr. S. Kovats. 721.174[3] cell line was kindly sent by Dr. Jean Petersen and Dr. Robert DeMars, University of Wisconsin, Madison, USA.

RJ2.2.5, BLS-1, SJO and 6.1.6 cell lines fall into four complementation groups (Table 1), i.e. hybridomas between groups complement each other, restoring HLA class II expression, whereas hybrids of cells belonging to the same group (possessing a defect in the same gene) remain HLA class II-negative[6–8, 20].

Cell culture. Cells were kept in suspension culture as described[14, 15]. Interferon γ (Genentech, USA) was added to cell cultures at 600 or 1000 μg/ml (final concentration) for 2 to 3 days. Then cells were harvested, washed and subjected to incubation with monoclonal antibodies for flow cytometry testing (see below).

Table 2. Reagents used for flow cytofluorimetry

Antibody	Specificity	Ig class
Anti-HLA class I:		
W6/32	HLA-A, B, C	IgG2a/κ
HB118 (clone PA2.6)	HLA-A, B, C	IgG1
HB82 (clone BB7.2)	HLA-A2	IgG2b
LN29	HLA-(B), C	
TU149	HLA-(B), C	IgG2a
Anti-HLA class II:		
L243	HLA-DR, unlabeled or phycoerythrin (PE)-conjugated	IgG2a
HB104 (clone 2.06)	HLA-DR	IgG1
B7/21	HLA-DP	IgG1/κ
Anti-B and -T cell markers:		
4G7	CD19	IgG1/κ
UCHT1	CD3	IgG1/κ
Anti-immunoglobulin sera, fluorescein isothiocyanate (FITC)-conjugated:		
RAMIG-FITC	Rabbit anti-mouse immunoglobulin	
GAMIG-FITC	Goat anti-mouse immunoglobulin	
GAHIG-FITC	Goat anti-human immunoglobulin	
Fluorochrome control:		
Str-PE	Streptavidin, phycoerythrin (PE)-conjugated	

Mouse monoclonal antibodies, other immunofluorescence reagents, and flow cytometry. Reagents used for flow cytometry are presented in Table 2. Mouse monoclonal antibodies were either kindly offered by Dr. Barbara Uchańska-Ziegler, Humboldt University, Berlin, Germany (antibody TU149), Professor Claudia A. Mueller, University of Tuebingen, Germany (LN29), and Dr. Giulio Spagnoli, Department of Research, Kantonsspital Basel, Basel, Switzerland (HB82, HB104, HB118), or purchased from Becton Dickinson (B7/21, 4G7, and unlabelled or phycoerythrin (PE)-conjugated L243), from Dako (W6/32 and fluorescein isothiocyanate (FITC)-conjugated UCHT1) and from Serotec (FITC-conjugated W6/32).

PE-conjugated streptavidin (Becton Dickinson) served as a negative control for PE-conjugated antibody L243. FITC-conjugated goat anti-human immunoglobulin serum (GAHIG-FITC, Behringwerke AG, Marburg, Germany) was used for detection of cell surface immunoglobulins. FITC-conjugated rabbit anti-mouse immunoglobulin serum (RAMIG-FITC) was used for detection of unlabelled antibodies bound to cells (indirect fluorescence).

Fluorochrome-conjugated monoclonal antibodies or GAHIG-FITC were used for direct staining of cells. For indirect immunofluorescence, cells were first incubated with unlabelled antibodies, washed, and incubated with FITC-RAMIG. Indirect and direct fluorescence and flow cytometry were performed following recommendations of Becton Dickinson and analyzed using FACScalibur flow cytofluorimeter and CellQuest software (Becton Dickinson).

Results

HAJ cells are HLA class II-negative similarly to BLS cell lines and in vitro class II deletion mutants

HAJ cells were negative with anti-HLA-DR antibody L243 as well as with anti-HLA-DP antibody (Fig. 1A, Fig. 2A and results not shown). Another anti-HLA-DR antibody, HB104, stained strongly PAJ cells but staining of HAJ was very weak (Fig. 1B) and presumably nonspecific, because 1) HLA-DR gene was not expressed in HAJ cells[14], 2) background staining with another antibody, HB82, directed to HLA-A2 antigen that is not present on HAJ cells[11, 14], was identical to HB104 staining (Fig. 1B), and 3) in other experiments, the antibody HB104 did not bind to HAJ cells over RAMIG-FITC control (e.g. see Fig. 4A below for comparison). This HLA class II-negative phenotype of HAJ cells was similar to that of BLS-derived cell lines BLS-1, BLS-2 and SJO, to BLS-like *in vitro* mutant cell lines RJ2.2.5 and 6.1.6, and to HLA class II region deletion mutant cell lines T2 and 721.174 (Fig. 2 and unpublished results). In all these experiments, class II positive B-LCLs Daudi and PAJ were strongly positive with anti-HLA-DR and anti-HLA-DP antibodies (Fig. 1 and Fig. 2).

HAJ cells express lower levels of HLA class I antigens than normal cell lines

HLA class I expression as revealed by flow cytometry with pan-class I antibody W6/32 and anti-HLA-C antibodies LN29 and TU149 was much weaker than on control PAJ cells, and it was frequently bimodal (Fig. 1A and 1B). Similar results were also obtained with another pan-class I antibody, HB118 (not shown). Class I expression on HAJ cells was also somewhat lower than on majority of BLS and BLS-like cell lines (Fig. 2A and data not shown here).

HAJ cell line is B lymphocyte-derived

The cell line HAJ was derived from an HLA class II-positive donor by transformation of peripheral blood lymphocytes by EBV *in vitro*[14]. Although, among leukocytes, the EBV most frequently infects and transforms B lymphocytes, the absence of HLA class II antigens from the surface of cells derived from HLA class II-positive donor suggested that in this particular case, the virus might have transformed another type of a cell. To test this assumption, we stained HAJ cells with antibodies to B lymphocyte surface markers, immunoglo-

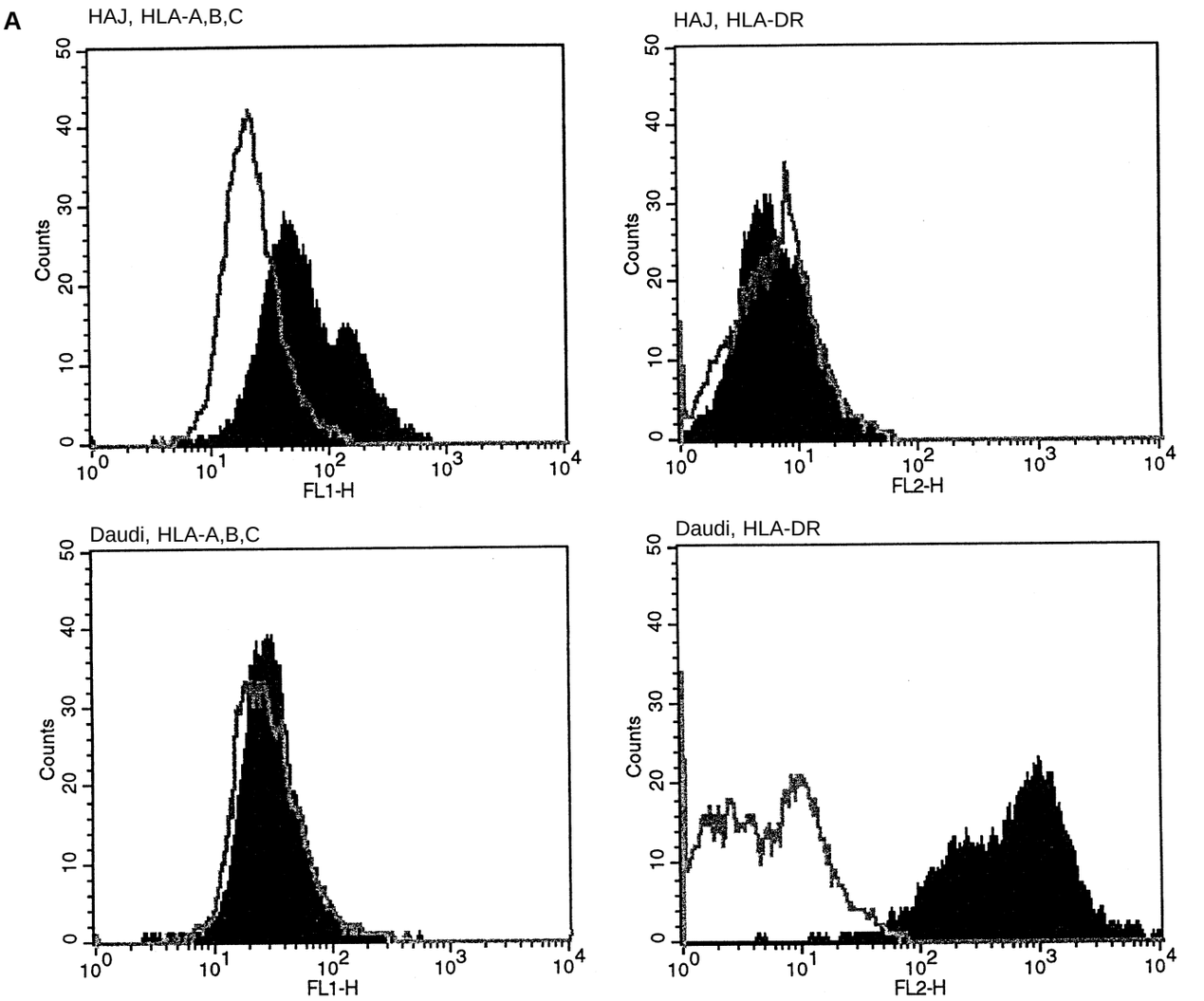
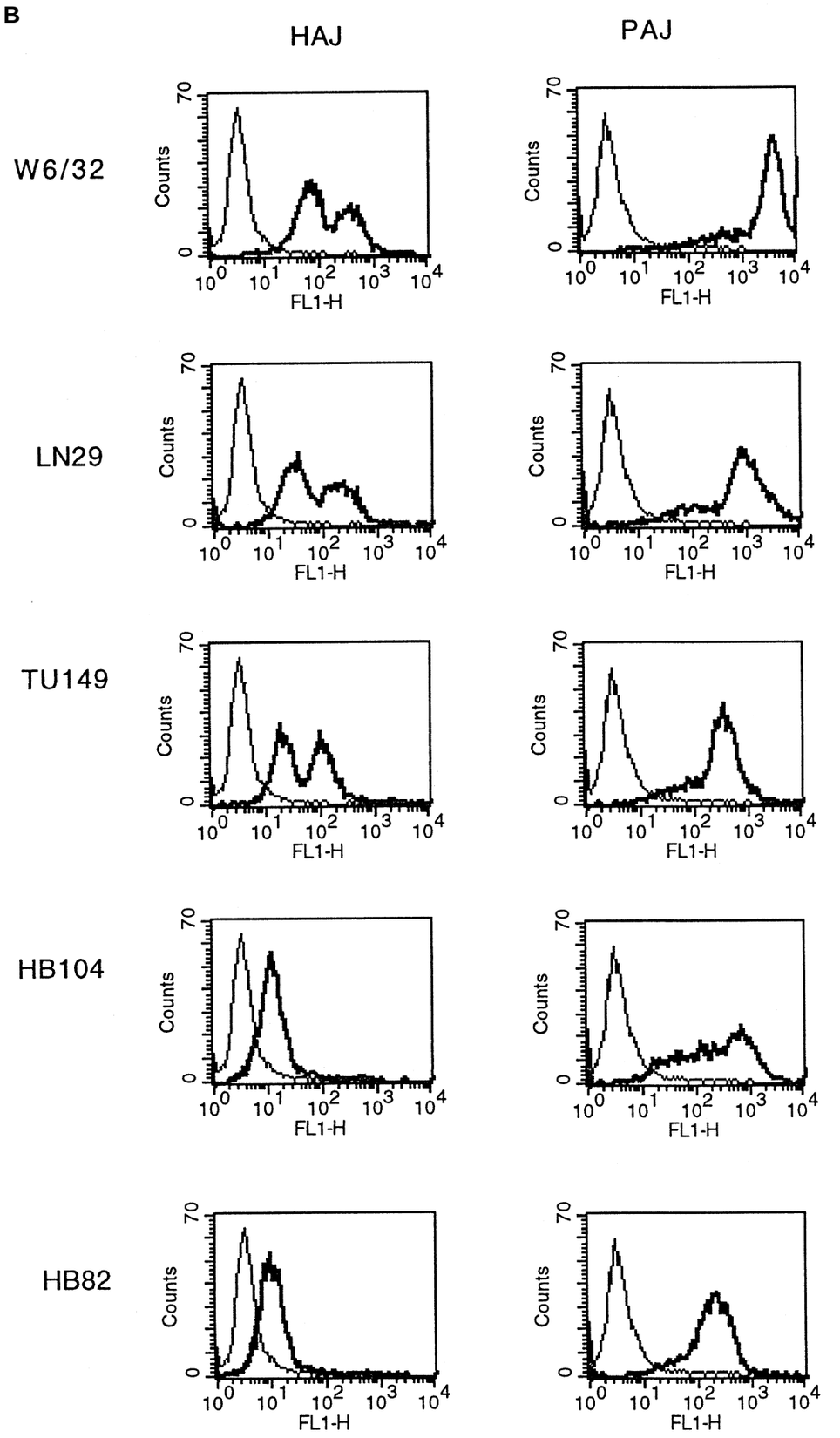


Fig. 1. HLA cell surface expression on HAJ cells. **A** – comparison of HLA expression on HAJ cells with that on class I-negative, class II-positive Daudi cells. HLA-A, B, C, antibody W6/32; HLA-DR, antibody L243. **B** – comparison of HLA expression on HAJ cells with that on normal HLA expressor PAJ cells. Bold line, antibodies: W6/32, anti-HLA-A, B, C; LN29 and TU149, anti-HLA-C; HB82, anti-HLA-A2; HB104, anti-HLA-DR. Thin line, RAMIG-FITC (negative control)


B
HAJ
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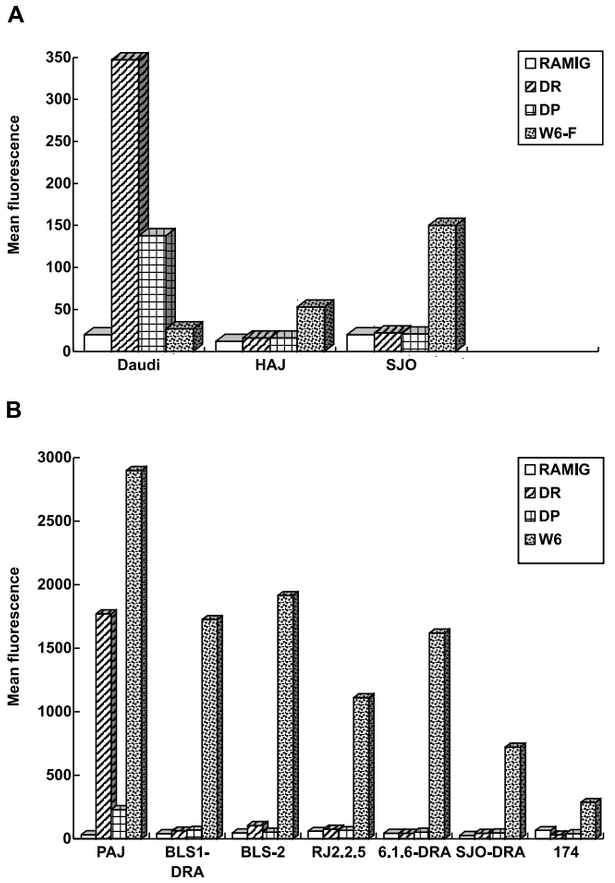

Fig. 2. HLA expression on HAJ cells and on BLS and BLS-like cell lines. The bars represent mean fluorescence. DR, antibody L243 (anti-HLA-DR); DP, antibody B7/21 (anti-HLA-DP); W6, antibody W6/32 (anti-HLA-A, B, C); RAMIG, i.e. RAMIG-FITC, negative control. **A** – comparison of HLA expression on HAJ cells with that on class I-negative, class II-positive Daudi cells and on BLS-derived, class I-low, class II-negative SJO cells. **B** – comparison of HLA expression on BLS and BLS-like cells with that on normal HLA expressor PAJ cells

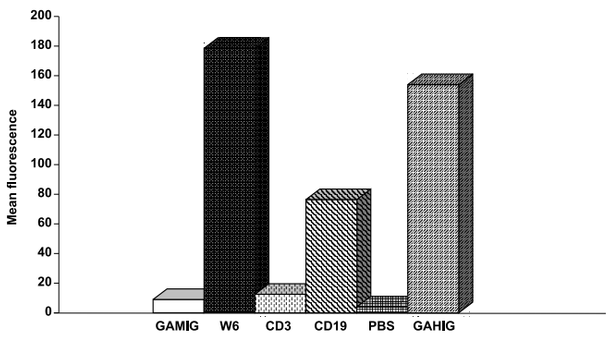

Fig. 3. HAJ cells display B lymhocyte but not T lymphocyte markers. First four bars: GAMIG-FITC alone (control) or with addition of W6/32, CD3 and CD19, as indicated in the legend. Last two bars: phosphor-buffered saline (control) or GAHIG-FITC

bulins (Ig) and CD19, as well as to the T lymphocyte antigen, CD3. As it is shown in Fig. 3, there was strong expression of surface Ig and CD19, but no CD3 was detected. Similar result was obtained with the control B-lymphoblastoid cell line PAJ (not shown). Thus, HAJ cells revealed a B cell phenotype.

IFN-γ upregulates class I HLA expression on HAJ cells but does not restore class II expression

Constitutive expression of class II antigen is limited to professional antigen-presenting cells: dendritic cells, B lymphocytes and activated macrophages. However, it may be induced on other cell types by IFN-γ[6, 20]. On the other hand, class I antigens are constitutively expressed on almost all cells in the body. Nevertheless,

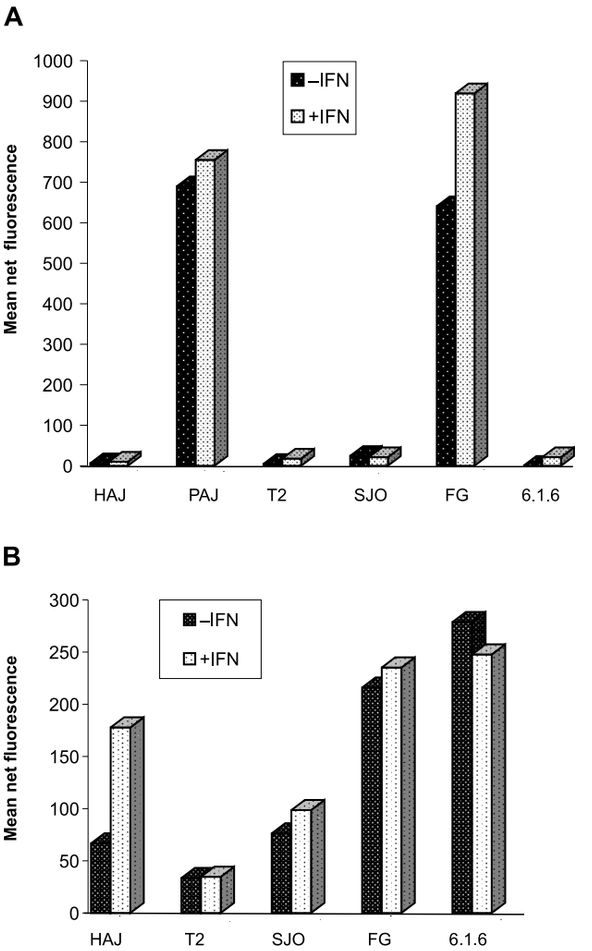

Fig. 4. Effect of IFN-γ pretreatment on HLA class II (**A**) and class I (**B**) expression on HAJ cells as compared with normal and mutant cell lines. Cells were cultured for 72 h with 600 U/ml IFN-γ (+IFN) or without it (–IFN), washed, stained with (**A**) anti-HLA-DR (L243) or (**B**) anti-HLA-A, B, C (HB118) antibody, and analyzed by flow cytofluorimetry. Similar results were obtained in two other experiments

this expression may also be upregulated by exposure to IFN-γ[7–9, 22, 23]. Since HAJ cells expressed no class II antigens and only low levels of class I antigens, we checked whether treatment with IFN-γ would restore class II and/or augment class I expression on HAJ cells. From 48 to 72 h culture in the presence of 600–1000 U/ml IFN-γ failed to induce class II expression on HAJ or BLS cell lines (Fig. 4A and data not shown). For obvious reasons, it also did not restore class II molecules on class II region deletant cell line T2 (Fig. 4A). Although there was a single report showing downregulation by IFN-γ of cell surface class II MHC antigens on mouse B lymphocytes[16], we observed rather opposite, if any, effect of this cytokine on class II-positive human B-LCLs PAJ and F. G. (Fig. 4A and data not shown).

In the same experiments, exposure to IFN-γ in two out of three experiments upregulated class I expression on HAJ cells; its effect on other cell lines was weaker and variable (Fig. 4B and experiments not shown).

Discussion

We found that HAJ cells lack HLA class II expression and exhibit low HLA class I expression on the cell surface. We established previously that cell surface and intracellular class II antigens are absent from HAJ cells because class II genes, albeit present, are not expressed[16, 17]. Thus, HAJ cells are phenotypically similar to cell lines derived from BLS patients. It should be emphasised that the cell line HAJ was derived from lymphocytes of an HLA class II- and class I-positive donor[16], and it originated from B lymphocyte and not from other, class II-negative cell lineage as evidenced by the presence of B lymphocyte markers and absence of T lymphocyte antigen CD3. Thus, the defect of class II expression must have happened during establishment of this cell line *in vitro*.

Low cell surface expression of HLA class I antigens on HAJ cells makes them similar to some BLS-derived cell lines such as SJO, and to HLA class II plus TAP deletion mutant cell line T2. Functional TAP molecule, encoded by *TAP1* and *TAP2* genes located between *HLA-DQ* and *HLA-DP* genes[21], is necessary for efficient loading of class I molecules with peptides, their stable complexing with β_2 microglobulin, and subsequent transport to the cell surface[13, 24]. The presence of *HLA-DRB1, -DQA-1* and *-DPA-1* genes in the HAJ genotype[12, 14] makes deletion of *TAP* gene(s) unlikely. Low constitutive expression but preserved capability to upregulate cell surface class I molecules upon stimulation with IFN-γ suggests that there is an additional regulatory mechanism whose defect in HAJ cells is responsible for low level of class I expression in the absence of IFN-γ. Interestingly, recent reports[7–9, 22, 23] indicate that class II transactivator (CIITA) molecule, originally thought to selectively influence class II gene expression (hence its name), actually enhances also class I expression. *CIITA* gene itself is also positively regulated by IFN-γ[17]. There are, therefore, two ways of class I upregulation by the IFN-γ: a "classical" one through interferon response sequence (IRE), and a "new" one mediated by CIITA[7, 8, 22, 23]. It was then conceivable that *CIITA* mutation was responsible both for the lack of class II and for the low level of class I expression on HAJ cells. Therefore, we attempted to fuse HAJ cells with hygromycin selective marker-transfected BLS and BLS-like cell lines belonging to four complementation groups shown in Table 1. These HAJ × BLS fusion experiments appeared not to be possible because HAJ cells displayed extremely high resistance to hygromycin similar to that of the transfectants[18]. However, transient hybridoma experiments revealed lack of functional CIITA in HAJ cells, locating them in the complementation group A (Dr. Barbara Lisowska-Grospierre, Hopital Necker, Paris, personal communication). As shown here, the defect of class II expression could not be compensated for by stimulation with IFN-γ, whereas this cytokine upregulated class I expression, presumably through the "classical" pathway independent of CIITA.

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