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# Analysis of Epstein–Barr virus (EBV) receptor CD21 on peripheral B lymphocytes of long-term EBV$^-$ adults

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## SUMMARY

Primary infections with EBV are rarely observed after the age of 20. Some individuals even remain seronegative all their lives. Previously, a lack of EBV receptors on B cells of persistently EBV$^-$ adults was described as a reason for long-term EBV-seronegativity. The present study examined the CD21 receptor status of 20 repeatedly EBV$^-$ healthy adults and 32 EBV$^+$ volunteers by means of flow cytometry. CD21 molecules on the surface of CD19$^+$ B cells were quantified using anti-IgG-coated microbeads. The percentage of CD19$^+$/CD21$^+$ B lymphocytes was slightly lower in the peripheral blood of EBV$^-$ donors, but the CD21 antibody binding capacity on CD19$^+$ B cells showed no significant differences between EBV$^-$ and EBV$^+$ adults. *In vitro* studies showed an equally good EBV transformability of peripheral B lymphocytes of EBV$^-$ and EBV$^+$ donors. Since HLA-DR was recently described as a co-receptor for EBV infection of B cells, we also determined HLA-DRB1 alleles in the EBV$^-$ group. We found a significant negative association of EBV-seronegativity with HLA-DR13 in comparison with 111 healthy blood donors. In summary, a biologically significant lack of the EBV receptor CD21 on peripheral B lymphocytes of persistently EBV$^-$ adults was excluded as a reason for long-term EBV-seronegativity.


**Keywords** Epstein–Barr virus Epstein–Barr virus receptors resistance CD21 HLA-DR

## INTRODUCTION

The human complement receptor type 2 (CR2), designed as CD21, is a membrane glycoprotein and serves as a receptor for the C3d, C3dg, and iC3b proteins of complement. Structurally, it consists of 15 or 16 extracellular short consensus repeats (SCR) followed by a transmembrane domain and an intracytoplasmic region [1]. CD21 was described as a receptor for the envelope glycoprotein gp350/220 of the EBV [2] and also as a receptor for interferon-alpha (IFN-$\alpha$) [3]. It is found on mature B lymphocytes and follicular dendritic cells [1]. Moreover, CD21-related antigens were suggested to be present on a minority of thymocytes and peripheral T cells [4]. Its presence on epithelial cells of the oropharynx also remains unclear, but it was shown that infection of the epithelium by EBV involves a receptor structurally homologous to CD21 [5]. The CD21 molecule is a functional ligand for CD23 and is involved in IgE and IgG4 production of human B lymphocytes [6]. Binding of the EBV antigen gp350/220 leads to capping of EBV–receptor complexes on the surface of B cells and mediates subsequent internalization of EBV into the cell [7].

EBV belongs to the human herpesvirus family and is ubiquitously distributed all over the world. It is the causative agent of infectious mononucleosis (IM) and is associated with several malignancies in man, such as African endemic Burkitt's lymphoma, nasopharynx carcinoma of the undifferentiated form, B cell lymphomas in immunosuppressed individuals, and Hodgkin's disease [8]. Infection with EBV commonly occurs during childhood in an asymptomatic form, leading to a rapid increase in EBV antibodies. Seroepidemiological data from various regions of the world demonstrate a prevalence of anti-EBV antibodies of 85–95% at the age of 20 years [9]. Thereafter, primary EBV infections are a rare event, but are still observed [10]. This suggests that some individuals remain EBV$^-$ due to natural resistance. In a previous study, we searched for immunological explanations for the phenomenon of long-term EBV-seronegativity and suggested a higher monokine production [11].

However, in 1981 Gervais *et al*. described a relative lack of EBV receptors on B cells of persistently EBV$^-$ adults [12]. In that study, B lymphocytes of 45·6% of the EBV$^-$ individuals were hardly able to bind EBV. This result was obtained by means of EBV-coupled, rosette-forming erythrocytes. Since this procedure

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is dependent on the researcher's subjective evaluation and is nowadays obsolete, we evaluated the expression of the meanwhile identified EBV receptor on B cells, CD21, of 20 long-term $EBV^-$ adults in comparison with 32 $EBV^+$ individuals by means of flow cytometry. The functionality of the detected CD21 receptor was assessed by EBV transformation studies. EBV infection of B cells also involves HLA-DR as a co-receptor [13]. To analyse the possible implications of different HLA-DR molecules in susceptibility to EBV, HLA-DRB1 genotyping was performed in the $EBV^-$ group by means of a polymerase chain reaction (PCR) method with sequence specific primers [14].

## SUBJECTS AND METHODS

*Study and control population*

A total of 1769 healthy blood donors had been screened for EBV-specific antibodies by means of a commercially available ELISA (Enzygnost Anti-EBV/IgG; Behring, Marburg, Germany) and by standard immunofluorescence technique (IFT; Fresenius, Oberursel, Germany). Of these, 30 adults lacking EBV-specific antibodies were investigated for their immunological properties as recently described [11]. After this first study, 20 of these $EBV^-$ adults (six women, 14 men, between 21 and 59 years old, median age 31 years) were reinvestigated in the present study and again found to be $EBV^-$ by IFT. Sera were screened for IgG antibodies to EB-viral capsid antigen (VCA) and EB nuclear antigen (EBNA) as well as for IgM antibodies to EB-VCA. EBV-seronegativity was defined with EB-VCA IgG < 1:40, EB-VCA IgM < 1:10, and EBNA IgG < 1:10. Blood samples from 32 volunteers (eight women, 24 men, between 20 and 52 years with a median age of 33 years) who were $EBV^+$ by IFT with EB-VCA $\geq$ 1:40, EB-VCA IgM < 1:10, and EBNA IgG $\geq$ 1 : 10 served as positive controls. ELISA and IFT were performed according to the manufacturer's recommendations.

*Analysis of B cells by flow cytometry*

EDTA anti-coagulated peripheral blood was drawn from the volunteers after informed consent. Differential blood counts for total numbers of leucocytes were obtained by automatic determination on a Coulter MAX-M cell counter (Coulter, Krefeld, Germany). After incubation of 100 $\mu$l blood each with 10 $\mu$l of anti-CD14/anti-CD45 (Mo2-RD1/KC56-FITC), 10 $\mu$l of anti-CD3/anti-CD19 (CD3(IgG1)-FITC/B4-RD1), and 10 $\mu$l of anti-CD3, anti-CD16, anti-CD56 (CD3-ECD, CD16-FITC, NKH-1-RD1) for 15 min, respectively, the samples were prepared for flow cytometry by an automatic workstation (Multi-Q-Prep; Coulter). Non-specific-binding isotype-specific MoAbs were used as controls (MsIg G2b-ECD/MsIgG1-RD1/MsIgG1-FITC, MsIgG2a-RD1, MsIgG1-FITC). All MoAbs were purchased from Immunotech/Coulter (Hamburg, Germany). For analysis we used a flow cytometer (EPICS XL-MCL; Coulter) equipped with an air-cooled argon laser (excitation wavelength 488 nm). After calculation of the total number of B cells per $\mu$l, the EDTA anti-coagulated blood was adjusted to 100 B lymphocytes/$\mu$l by the use of PBS pH 7·2 (GIBCO, Paisley, UK). Blood/PBS was then stained with 10 $\mu$l of anti-CD19 and anti-CD21 each (B4-RD1, CD21-FITC), incubated for 15 min and afterwards prepared with the Multi-Q-Prep workstation. Anti-CD21 MoAb was from clone BL13 [15]. IgG1-FITC/IgG1-PE was used as an isotype-specific control. The samples were assayed at a flow rate of 1000 particles/s, for an analysis of 5000 B cells per sample. The lymphocyte population was gated in an electronic bit map by using the forward scatter *versus* the log90° scatter (side scatter $\log_{10}$). The gated population was further analysed in a correlation plot of CD19-PE *versus* CD21-FITC. B lymphocyte-specific antibody binding was calculated by electronic subtraction of non-specific binding of the isotypic control from the binding of the test sample and expressed as a percentage of antibody-positive cells and as mean channel fluorescence.

*Quantification of the antibody binding capacity*

We quantified the B lymphocyte-specific CD21 antibody binding after calibration with goat anti-mouse IgG-coated microbeads (Quantum Simply Cellular; FCSC Europe, Leiden, The Netherlands) following a recently described protocol [16]. Similar protocols are now widely used for quantification of surface markers on leucocytes [17–20]. The microbead suspension contained four bead populations with different defined capacities to bind murine MoAbs and a blank control. The beads were all of the same size (7–10 $\mu$m) according to the size of lymphocytes. Briefly, 50 $\mu$l of microbead suspension were incubated with 20 $\mu$l of FITC-conjugated anti-CD21 MoAb. Incubation and flow cytometric analysis were carried out under the same instrument settings as for the B lymphocyte analysis. Data analysis was performed with special software enclosed with the microbeads (QuickCal Program; FCSC). By determination of the peak fluorescence signal for each microbead population (Fig. 1), a calibration curve with the relative level of fluorescence per binding site was calculated (Fig. 2). The number of binding sites on the investigated B cells was then calculated from the mean channel fluorescence signals obtained with the anti-CD21 staining of B cells.

*Preparation of infective EBV*

B95-8 marmoset cell line was grown in culture medium (IMDM; BioWhittaker, Verviers, Belgium; supplemented with 10% heat-inactivated fetal calf serum (FCS; GIBCO), L-glutamine 2 mM (GIBCO), penicillin 100 U/ml, streptomycin 100 $\mu$g/ml (GIBCO), 24 ml minimal essential medium-alpha, (GIBCO), 5 ml OPI media

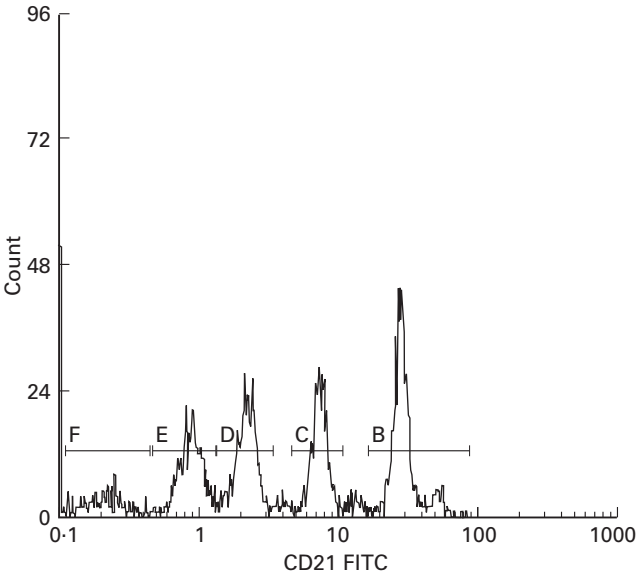


**Fig. 1.** Fifty microlitre microbead suspensions were incubated with 20 $\mu$l of anti-CD21(FITC) MoAb. Four different populations and a blank control are distinguishable (B–F).

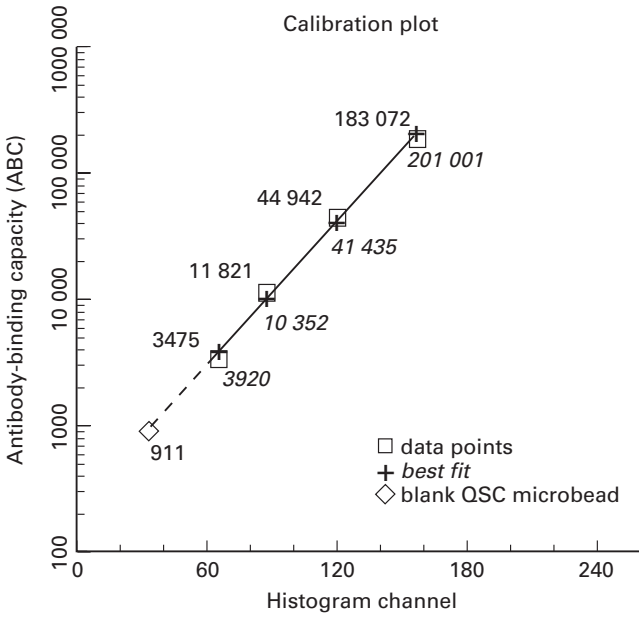


**Fig. 2.** Relationship between the amount of anti-mouse IgG bound to the microbeads (antibody-binding capacity) and the mean channel fluorescence signal of each microbead population and the blank control incubated with anti-CD21(FITC) (calculated antibody-binding capacity); correlation $r^2 = 0{\cdot}995$.

supplement (Sigma, Deisenhofen, Germany)) for 10 days at a density of $5 \times 10^5$ cells/ml. Cells and medium were then frozen at −20°C for 2 h to release intracellular virus particles. Afterwards, cell suspension was filtrated through a sterile 0·45-$\mu$m filter, aliquoted in 1-ml portions and stored at −80°C. The virus preparation had a titre of $10^{-3}$ transforming units as determined on human umbilical cord blood lymphocytes (HUCBL). Ten replicate aliquots each containing $2 \times 10^5$ HUCBL were infected with a 10-fold dilution series of the EBV stock solution. Efficiency of transformation was defined as the negative log to the base 10 of the virus dilution which induced immortalization in at least 50% of the cultures after 4 weeks.

*EBV transformation*

Peripheral blood mononuclear cells (PBMC) of six persistently EBV$^-$ and six age- and sex-matched EBV$^+$ adults were isolated from buffy coats by standard density centrifugation (Ficoll Separation Solution; Biochrom, Berlin, Germany). After two wash steps in PBS (10 min at 400 ***g***) cells were resuspended in wash medium (RPMI 1640 (Biochrom), 2% FCS, L-glutamine 2 mM, penicillin 100 U/ml, streptomycin 100 $\mu$g/ml) and adjusted to a concentration of $1 \times 10^7$/ml. To eliminate lysosome-rich, inhibiting cell populations, L-leucyl-L-leucine-methylester (2·5 mM; Bachem, Heidelberg, Germany) was added to $1 \times 10^8$ PBMC. Following 15 min of incubation at room temperature and two wash steps with wash medium, cells were resuspended in 2 ml of the EBV stock solution. A negative control consisting of culture medium was included in every experiment. After removing excess virus by another wash step, 10 replicate aliquots containing $2 \times 10^6$ cells in 2 ml were dispensed into 24-well tissue culture plates (Becton Dickinson, Lincoln Park, NJ) and incubated for 4 weeks in a humidified atmosphere with 5% $CO_2$. Cultures were fed weekly by carefully removing half of the supernatant and adding 1 ml of fresh culture medium. Immortalization was assessed visually by means of an inverted microscope and expressed as the percentage of cultures showing typical lymphoblastoid cell clumps.

*DNA typing of HLA-DRB1*

DNA was extracted from PBMC by proteinase K digestion and a salting-out procedure. DNA was adjusted to a concentration of 100 $\mu$g/ml and used at a final concentration of 50 ng per reaction. We used our protocol for rapid DNA typing with nested PCR and sequence specific primers as published by Bein *et al*. [14]. This protocol dissolves HLA-DRB1 alleles at the serologically determinable level (low resolution). The results were compared with frequencies of HLA-DRB1 alleles in our community [21].

*Statistical analysis*

Statistical analysis was performed with commercially available software for personal computers (SPSS for Windows 5.01). Data are expressed either as mean ± s.d. or as median. Comparison of EBV$^-$ and EBV$^+$ individuals regarding flow cytometry was carried out with the Mann–Whitney *U*-test. HLA association was calculated with the $\chi^2$ test. $P < 0{\cdot}05$ was considered significant.

## RESULTS

*Cell counts*

With regard to the blood cell counts, we found a significantly higher percentage of monocytes in the peripheral blood of EBV$^-$

**Table 1.** Differential blood cell counts and lymphocyte subpopulations of EBV$^-$ and EBV$^+$ adults

| | EBV$^-$ ($n = 20$) | | EBV$^+$ ($n = 32$) | |
|---|---|---|---|---|
| *Differential blood cell counts* | | | | |
| Leukocytes/$\mu$l | | 5370 ± 1082 | | 5981 ± 1729 |
| Neutrophils (%; $\mu$l) | 57·4 ± 10·8 | 4515 ± 970 | 55·3 ± 7·5 | 3384 ± 1181 |
| Lymphocytes (%; $\mu$l) | 27·7 ± 8·4 | 1555 ± 448 | 31·3 ± 7·6 | 1831 ± 590 |
| Monocytes (%; $\mu$l) | 9·7* ± 4·4 | 515 ± 157 | 8·0* ± 2·3 | 519 ± 175 |
| *Lymphocyte subpopulations* | | | | |
| CD3 T cells (%; $\mu$l) | 71·7 ± 5·8 | 1138 ± 377 | 74·6 ± 5·4 | 1370 ± 460 |
| CD19 B cells (%; $\mu$l) | 14·3 ± 3·7 | 221 ± 79 | 12·7 ± 4·1 | 232 ± 103 |
| CD16, CD56 NK cells (%; $\mu$l) | 6·6 ± 4·1 | 121 ± 64 | 6·2 ± 3·3 | 135 ± 76 |

Values per $\mu$l are mean ± s.d.; percentages are median ± s.d.
*$P < 0{\cdot}05$.

**Table 2.** $CD21^+$ B lymphocytes of long-term $EBV^-$ and $EBV^+$ adults

| $CD21^+$ B lymphocytes | $EBV^-$ ($n = 20$) | $EBV^+$ ($n = 32$) |
|---|---|---|
| Per $\mu$l | 191 ± 76 | 209 ± 93 |
| Percent of lymphocytes | 12·4 ± 3·3 | 11·4 ± 3·5 |
| Percent of B lymphocytes | 86·0* ± 10·4 | 92·6* ± 9·2 |

Values per $\mu$l are mean ± s.d.; percentages are median ± s.d.
*$P < 0{\cdot}05$.

adults ($P < 0{\cdot}05$). All other leucocyte values were within the normal range (Table 1). The distribution of the lymphocyte subpopulations showed no significant differences between the two study populations.

*Determination of $CD21^+$ cells*

After adjusting the peripheral blood to 100 B cells/$\mu$l, we determined the absolute number of $CD19^+/CD21^+$ cells/$\mu$l and the percentage of $CD21^+$ B lymphocytes in relation to all lymphocytes as well as in relation to all B lymphocytes ($CD19^+$). As shown in Table 2, we observed differences neither in total numbers of $CD21^+$ B cells nor in relation to all lymphocytes. However, a significantly lower percentage of CD21-expressing B cells relative to all B cells was found. By means of the anti-CD21 MoAb, clone BL13 [15], no expression of CD21 on peripheral T cells was seen (data not shown).

*Quantification of the CD21 binding capacity on B cells*

Determination of CD21 binding sites on peripheral B cells from $EBV^-$ and $EBV^+$ donors was carried out within a period of 3 weeks. A new calibration curve was created each day by means of the microbead suspension. We found a significantly lower CD21-FITC mean fluorescence signal ($P < 0{\cdot}01$) on B cells of $EBV^-$ individuals (Fig. 3). However, the calculated CD21 antibody binding capacity on peripheral $CD19^+$ B cells did not differ significantly between $EBV^-$ and $EBV^+$ adults after calibration with the anti-IgG-coated microbeads.

*EBV-induced transformation*

To assess the susceptibility of $EBV^-$ adults to EBV infection, we determined the *in vitro* transformability of peripheral B cells of six persistently $EBV^-$ donors and six age- and sex-matched $EBV^+$ controls. As given in Table 3, cultures of either group showed an overall good transformability with a median efficiency of transformation of 80% in either group.

*HLA-DRB1 frequencies*

We performed a low resolution HLA-DRB1 genotyping of 20 $EBV^-$ adults. The absolute as well as the relative HLA-DRB1 antigen frequencies are given in Table 4. The results were compared with data from a large group of healthy blood donors regardless their EBV serostatus ($n = 111$) who were analysed by high-resolution HLA-DR typing [21]. A significant negative association was observed for HLA-DR13 and EBV-seronegativity ($\chi^2 = 6{\cdot}42$; $P < 0{\cdot}05$). No other HLA-DR molecule revealed a positive or negative association with EBV-seronegativity.

## DISCUSSION

A relative lack of EBV receptors on B cells of persistently $EBV^-$ adults as a reason for long-term EBV-seronegativity was suggested in a previous study [12]. That study examined the presence of EBV receptors in 66 $EBV^-$ or $EBV^+$ adults by means of EBV-coupled, rosette-forming erythrocytes and found a relative lack of EBV receptors in 45·8% of the $EBV^-$ group (11 of 24), but in no $EBV^+$ individual, i.e. the percentage of B cells able to bind EBV was < 2% in the $EBV^-$ group. The lack of EBV receptors showed an overall presence of < 0·5% in relation to the whole population of

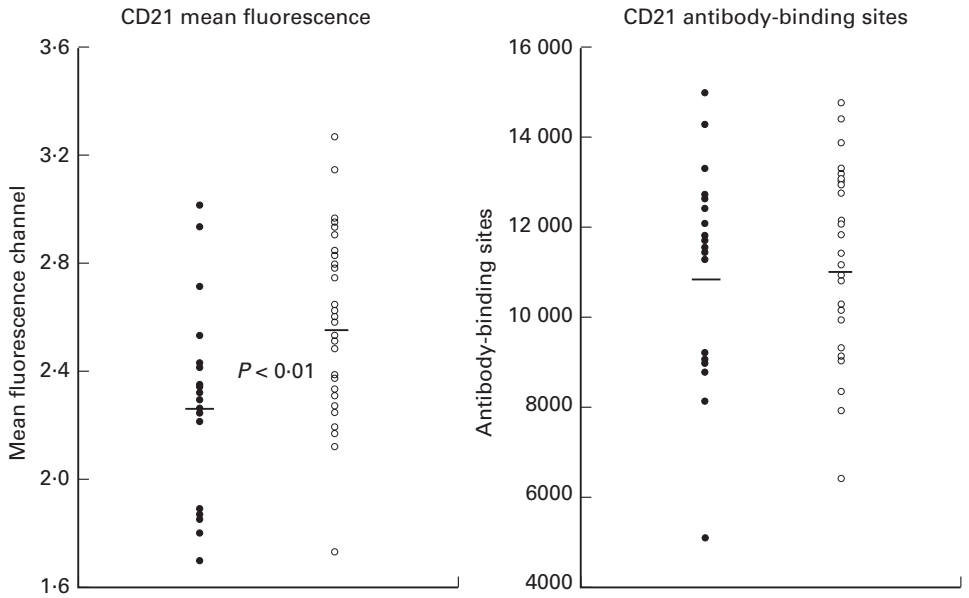


**Fig. 3.** Mean channel fluorescence signals of CD21-FITC-stained B cells as well as the CD21 antibody-binding capacity on B cells of 20 $EBV^-$ and 32 $EBV^+$ adults. Antibody binding was calculated by calibrating the fluorescence signals against the microbead standard. ●, $EBV^-$, $n = 20$; ○, $EBV^+$, $n = 32$.

**Table 3.** EBV-induced transformation in long-term cultures of $EBV^-$ and $EBV^+$ adults

| Age- and sex-matched donors | Efficiency of transformation (in percentage of positive cultures) | |
|---|---|---|
| | $EBV^-$ | $EBV^+$ |
| 1 | 100 | 100 |
| 2 | 100 | 100 |
| 3 | 30 | 100 |
| 4 | 20 | 50 |
| 5 | 80 | 80 |
| 6 | 100 | 80 |

Efficiency of transformation is expressed as the percentage of 10 replicate cultures which showed typical EBV-transformed cell clumps after 4 weeks of culture. Peripheral blood mononuclear cells (PBMC) of each donor pair were isolated and infected with EBV under similar conditions on the same day.

> 2000 individuals. However, these data have not been confirmed yet. Therefore, we examined the presence of the now known EBV receptor CD21 on B lymphocytes of repeatedly $EBV^-$ adults by means of flow cytometry and MoAbs.

Peripheral blood cell analysis revealed a significantly higher monocyte count in $EBV^-$ adults. This is consistent with our recent findings about immunological properties of $EBV^-$ adults [11]. The result underlines our consideration of higher monocyte activity in maintenance of EBV-seronegativity.

**Table 4.** Frequencies of HLA-DRB1 antigens

| Antigens | $EBV^-$ adults ($n = 20$) | | Reference group ($n = 111$)† | |
|---|---|---|---|---|
| | Absolute | Percentage | Absolute | Percentage |
| DR1 | 5 | 12·5 | 22 | 9·9 |
| DR4 | 5 | 12·5 | 36 | 16·2 |
| DR7 | 6 | 15·0 | 27 | 12·2 |
| DR8 | 0 | 0·0 | 7 | 3·2 |
| DR9 | 0 | 0·0 | 2 | 0·9 |
| DR10 | 0 | 0·0 | 3 | 1·4 |
| DR11 | 7 | 17·5 | 26 | 11·7 |
| DR12 | 3 | 7·5 | 8 | 3·6 |
| DR13* | 1 | 2·5* | 31 | 14·0* |
| DR14 | 1 | 2·5 | 5 | 2·3 |
| DR15 (2) | 6 | 15·0 | 34 | 15·3 |
| DR16 (2) | 0 | 0·0 | 3 | 1·4 |
| DR17 (3) | 6 | 15·0 | 18 | 8·1 |
| DR18 (3) | 0 | 0·0 | 0 | 0·0 |
| Σ | 40 | 100 | 222 | 100 |

HLA-DRB1 genotyping was performed by polymerase chain reaction (PCR) amplification with sequence-specific primers [14]. Allele numbers in parentheses refer to the old DR nomenclature.

†Data of the reference group were obtained by high-resolution DNA typing of Caucasian blood donors regardless of their EBV serostatus [21].

* $\chi^2 = 6{\cdot}42$; $P < 0{\cdot}05$.

Determination of the number of CD21-expressing B lymphocytes in our study showed a significantly lower percentage in $EBV^-$ individuals. Besides follicular dendritic cells, CD21 is expressed only on mature B lymphocytes [1]. This might suggest that $EBV^-$ persons contain more immature B lymphocytes in their blood. However, it is rather unlikely that the observed difference in the number of CD21-expressing B cells between $EBV^-$ and $EBV^+$ adults in fact reflects the relative lack of EBV receptors described by Gervais *et al*. [12]. Since $EBV^-$ adults still show a high number of $CD21^+$ B cells (86·0%), it is doubtful whether this 'lack' has any relevance regarding the prevention of EBV infection. At least our transformation experiments demonstrated in principle the infectability of B lymphocytes of $EBV^-$ adults. The biological relevance of the significant reduction in $CD21^+$ B cells of $EBV^-$ individuals is therefore questionable.

Moreover, quantification of the mean CD21 binding capacity of B lymphocytes showed no significant differences between $EBV^-$ and $EBV^+$ individuals. The findings by Gervais *et al*. [12] are thus probably not due to a significant lack in surface CD21 molecules of $EBV^-$ adults' B cells. Interestingly, we found a significantly lower CD21-FITC mean channel fluorescence signal for $EBV^-$ in comparison with $EBV^+$ adults. This underlines the importance of calibrating the fluorescence signal against a known standard if the experiments aim at a quantification of surface molecules, and investigations are carried out over a period of several days.

One might argue that the CD21 MoAb used in our study only detects an EBV receptor which is unable to bind EBV or to mediate virus penetration into the cell. The extracellular part of the CD21 molecule consists of 15 or 16 SCR domains [1]. The binding sites for C3d, EBV and IFN-$\alpha$ are all localized in the first two SCR [1,3]. A deletion of this region would presumably lead to a CD21 molecule unable to bind EBV. The anti-CD21 MoAb we used does not bind within the first two SCR domains [15]. The possible expression of a truncated form of CD21 lacking the first two SCR is thus not completely ruled out in persistently $EBV^-$ individuals. However, two observations might argue against this consideration: (i) the only splicing variant of CD21 described so far is the lack of exon 11 resulting in 15 instead of 16 SCR [22]; (ii) peripheral B lymphocytes from $EBV^-$ adults were as easily infectable by EBV as those from $EBV^+$ donors, suggesting the presence of an active CD21 receptor. Nevertheless, a CD21 molecule exhibiting a mutation within the EBV binding region—comparable to the effect of chemokine receptor 5 gene mutation in resistance to HIV-1 [23]—is still conceivable for some persistently $EBV^-$ adults and needs further investigation.

Because EBV is known to bind to HLA-DR [24] which is constitutively present on B lymphocytes, and because HLA-DR serves as a cofactor for EBV infection of B cells [13], an association for HLA-DR molecules and the susceptibility to EBV infection appeared possible. Data concerning HLA-molecules of $EBV^-$ adults was published in 1981 [12,25]. These groups only investigated the expression of HLA class I alleles, since determination of class II alleles was highly inaccurate at that time. They described inconsistent and contradictory associations regarding HLA-A and -B alleles, neither of which could be confirmed by us (data not shown). However, HLA-DRB1 typing in 20 repeatedly $EBV^-$ adults for the first time showed a significant negative association for HLA-DR13 and EBV-seronegativity. Binding of EBV to HLA-DR involves an outer envelope glycoprotein of EBV, gp42 [24]. EBV lacking gp42 is not able to infect

B cells [26]. Although gp42 probably binds to the constant part of the HLA-DR $\beta$-chain [13], it might be possible that EBV has a higher affinity to HLA-DR13 which is of relevance for infection of B cells.

In conclusion, the presented data reject the suggested lack of EBV receptors on B cells of persistently EBV⁻ adults. A negative association was found between EBV-seronegativity and presence of HLA-DR13. The impact of this finding has to be studied further, but its importance is doubtful, since it concerns only a minor fraction of EBV⁻ individuals.

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