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EBV-Induced Expression and HLA-DR-Restricted Presentation by Human B Cells of αB-Crystallin, a Candidate Autoantigen in Multiple Sclerosis[1]

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The development of multiple sclerosis is most likely influenced by autoimmune responses to central nervous system myelin proteins as well as by infections with common viruses such as EBV and human herpesvirus-6. However, much remains to be established on how these factors interact. In this study, we show that upon EBV infection, human B cells start to express αB-crystallin, a small stress protein that was identified previously as an immunodominant Ag of CNS myelin in multiple sclerosis patients. EBV-induced expression of αB-crystallin in B cells leads to HLA-DR-restricted presentation of the protein and to activation of proinflammatory αB-crystallin-specific Th cells. While αB-crystallin is present in EBV-infected human B cells, the protein is absent from human lymphoid tissues under normal conditions. This is in sharp contrast to other stress proteins such as heat-shock protein (hsp)27 and hsp60 that are ubiquitously expressed in these tissues. In addition, the absence of αB-crystallin from lymphoid tissues in humans is unique as compared with other mammals. All other species examined, including rodents, sheep, and primates, showed constitutive expression of αB-crystallin in secondary lymphoid tissues and sometimes even in the thymus. Since constitutive lymphoid expression most likely results in immunologic tolerance, such a state of tolerance to αB-crystallin can be expected for all of these species, but not for humans. When taken together, our data provide evidence for a novel mechanism by which common viral infections can trigger myelin-directed autoimmunity in a way that is unique for humans. ***The Journal of Immunology*****, 1999, 162: 129–135.**

Multiple sclerosis (MS)[3] is characterized by the development of foci of demyelinating and axon-damaging inflammation in human central nervous system (CNS) white matter (1, 2). Although the etiology of MS is still largely unknown, its development is likely to be determined by infectious events as well as by autoimmunity to CNS myelin components. Infectious events contribute to the pathogenesis of MS not only in affecting disease susceptibility, but also in triggering relapses once autoimmunity has manifested itself (3–5). Viruses including EBV, measles (MV), rubella, mumps, and human herpes virus-6 (HHV-6) have been proposed to be involved in the development of MS (6–14). However, no convincing evidence for a single causative virus in MS has been reported to date.

The target Ag(s) of the autoimmune response in MS is still subject of extensive study. In view of the restricted localization of MS lesions to CNS white matter, it is likely that pathogenic T cells are locally triggered by one or more components of the myelin sheath. Previously, we have shown that in myelin from the brains of MS patients the stress protein αB-crystallin serves as an immunodominant autoantigen to human T cells (15). Especially at the early active stages of inflammation and myelin breakdown in the CNS during the formation of MS lesions, the oligodendrocyte/myelin unit contains elevated levels of αB-crystallin (16).

αB-Crystallin is a member of the family of small heat-shock proteins (hsp) (17). Previous studies have indicated that the in vivo expression of αB-crystallin is different from many other stress proteins, including family members of hsp60, hsp70, and hsp90, as well as small stress proteins such as hsp27 (18–21). As a rule, most stress proteins are expressed ubiquitously in vertebrates, and they exert routine housekeeping functions during protein biosynthesis and protein transport across membranes. Stress-induced accumulation of, for example hsp60 and hsp70, has been documented for a large variety of cell types and for many different types of stress. In contrast, constitutive expression of αB-crystallin has only been found in a restricted number of tissues, including the eye lens and cardiac and skeletal muscle, while low-level expression has been reported for renal epithelial cells, lung tissue, and CNS glia cells; in most other tissues, αB-crystallin is undetectable (22–24). Stress-induced expression of αB-crystallin appears to be similarly restricted. In humans, stress-induced accumulation of αB-crystallin has been documented for only a limited number of disorders, notably neurodegenerative diseases, neurotropic infections, and some malignancies (16, 20, 25–28). Stress-induced accumulation of αB-crystallin is particularly well documented for human and rodent astrocytes or cell lines derived thereof (29, 30).

In this study, we show that contrary to hsp27 and hsp60, αB-crystallin is absent from healthy human lymphoid tissues, including spleen, thymus, and PBL. In contrast, healthy lymphoid cells from several other mammalian species, including rodents, sheep, and primates, contain readily detectable levels of αB-crystallin.

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[3] Abbreviations used in this paper: MS, multiple sclerosis; CBMC, cord blood mononuclear cell; CNS, central nervous system; EBV-LCL, EBV-transformed lymphoblastoid cell line; HHV-6, human herpes virus-6; hsp, heat-shock protein; MV, measles virus; TCL, T cell line.

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Infection of human peripheral blood B cells with EBV results in the expression of αB-crystallin and its HLA-DR-restricted presentation to human T cells. The activated T cells are proinflammatory Th1 cells in that they secrete large amounts of IFN-γ, but little, if any, IL-4. When taken together, these data indicate that virus-induced presentation of αB-crystallin by human B cells is exceptional since it is likely to occur in a nontolerized background. The resulting T cell response is cross-reactive with CNS myelin (15) and, therefore, may well contribute to the development of MS.

Materials and Methods

Animal and human material

Human PBMC from healthy donors were obtained from the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service (Amsterdam, The Netherlands). PBMC from MS patients, diagnosed as having definite MS, were obtained through Dr. C. H. Polman (Department of Neurology, Free University Hospital, Amsterdam, The Netherlands). Cord blood mononuclear cells (CBMC) were kindly supplied by Dr. C. J. P. Boog, and human spleen by Dr. N. M. Lardy (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service). Human thymus was provided by Dr. M. Verschuren (Erasmus University, Rotterdam, The Netherlands). EBV-negative B cell lymphomas of patients diagnosed with monoclonal gammopathy were kindly provided by Dr. J. W. Gratama (The Daniel den Hoed Clinic, Rotterdam, The Netherlands).

Female SJL mice were obtained from Erasmus University. Female BALB/cbyJIco (BALB/c), C57BL/6JIco (C57BL/6J), and 129/SvPasIco (129/Sv) mice were obtained from the Broekman Institute (Someren, The Netherlands). Male Lewis/CrIBr (Lewis) rats were purchased from Charles River (Wilmington, MA)/the Broekman Institute. Female C57BL/6KH mice and male BN/BiRy (Brown Norway) rats were bred in our animal facilities. Peripheral blood of a sheep was a kind gift of veterinary surgeon Dr. P. Vugts (Oud-Gastel, The Netherlands). Peripheral blood samples of various primates were kindly provided by Dr. B.'t Hart and H. Brok (Biomedical Primate Research Center, Rijswijk, The Netherlands).

Single cell suspensions of thymus, spleen, and cardiac muscle were prepared. PBMC and splenocytes were isolated by density-gradient centrifugation. Cells were washed twice with PBS, frozen in liquid nitrogen, and stored at −70°C until RNA isolation was performed.

Generation of αB-crystallin-specific T cell lines

PBMC from either healthy control subjects or MS patients were cultured in RPMI 1640 (Dutch modification) supplemented with 100 U · ml^{-1} penicillin, 0.1 mg · ml^{-1} streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES (pH 7.4), and 5% (v/v) pooled human serum at 2×10^5 cells per 100 μl in round-bottom wells at 37°C in the presence of 5 μg · ml^{-1} αB-crystallin. αB-Crystallin was purified from bovine eye lenses by reversed-phase HPLC (15). On days 5 and 8, 10% (v/v) Lymphocult-T (Biotest Seralc, Zaventem, Belgium) was added as a source of growth-promoting cytokines including IL-2. Ag specificity was evaluated by split-well assays on day 11 or 12. Specific T cell lines (TCL) were kept in culture by weekly stimulation with 10^6 ml^{-1} irradiated (30 Gy) autologous PBMC in the presence of 5 μg · ml^{-1} αB-crystallin for at least 7 wk. Bovine αB-crystallin is identical to the human homologue, except for conservative substitutions at 4 of the 175 amino acid positions.

T cell proliferation assays

Proliferation assays were performed by seeding 5×10^4 T cells with 10^5 irradiated (30 Gy) autologous PBMC and varying doses of αB-crystallin in 200 μl medium in round-bottom wells. After 72 h of culture, 20 kBq [^{3}H]thymidine (Amersham Life Sciences, Arlington Heights, IL) was added per well, and, after another 16 h, thymidine incorporation was determined by using a beta plate counter (Canberra Packard, Meriden, CT). To determine TCL responses using EBV-LCL as APC, TCL were seeded at 5×10^4/well in the presence of 5×10^4 irradiated (50 Gy) autologous EBV-LCL as APC and varying doses of purified αB-crystallin. The effect of anti-HLA Abs on TCL responses was examined by proliferation assays in the presence of 10 μg · ml^{-1} αB-crystallin. APC were preincubated for 30 min with mAbs B8.11.2 (anti-HLA-DR, IgG2b), SPV-L3 (anti-HLA-DQ, IgG2a), or B9.12.1 (anti-HLA-class I) (31).

Flow cytometry

The expression of various surface Ags on αB-crystallin-specific TCL was analyzed by direct immunofluorescence dual staining. For this purpose, T cells were incubated with mAbs directed at CD3, CD4, CD8, αβ-TCR, or γδ-TCR conjugated with FITC or phycoerythrin (Becton Dickinson) and analyzed on a FACScan flow cytofluorometer using Cell Quest software (Becton Dickinson).

Cytokine assays

To examine cytokine release by TCL in response to αB-crystallin, 5×10^5 T cells were seeded with 10^6 autologous, irradiated PBMC and 5 μg αB-crystallin in 1 ml of culture medium. After 3 days, culture supernatants were examined by ELISA for the presence of IFN-γ (Pelikine Compact, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service) and IL-4 (32).

RT-PCR

mRNA was isolated using the RNAzol B method (Campro Scientific, Veenendaal, The Netherlands) and isopropanol precipitation. Using 1 μg of mRNA as a template, copy DNA was produced using the Reverse Transcription System (Promega, Madison, WI). For amplification, cDNA (1 μl) was added to 1 μl 10 mM dNTP mix, 5 μl 10× *Taq* polymerase buffer (500 mM KCl, 600 μg · ml^{-1} BSA, 100 mM Tris-HCl (pH 8)), and 1 unit *Taq* DNA polymerase (Life Technologies, Amsterdam, The Netherlands) in a final volume of 50 μl. Primers and amplification conditions were as follows: αB-crystallin, 5′-AGCTGGTTTGACACTGGACT-3′ and 5′-CAATTCAAGAAAGGGCATC-3′, 35 cycles of melting at 94°C, annealing at 65°C, and extension at 72°C for 30 s each in the presence of $MgCl_2$ at a final concentration of 3 mM; β-actin, 5′-AAGATGACCCAGATCATGTTTGAG-3′ and 5′-AGGAGGAGCAATGATCTTGATCTT-3′, 35 cycles of melting at 94°C, annealing at 65°C, and extension at 72°C for 30 s each in the presence of $MgCl_2$ at a final concentration of 2.25 mM; Hsp27, 3′-CAAAAGAACACACAGGTGCG-5′ and 3′-TCCCTGGATGTCAACCACTT-5′, 40 cycles of melting at 94°C, annealing at 60°C, and extension at 72°C for 60 s each in the presence of $MgCl_2$ at a final concentration of 1.5 mM; Hsp60, 3′-TTGGGCTTCCTGTCACAGTT-5′ and 3′-TTCGATGCATTCCAGCCTTG-5′, 40 cycles of melting at 94°C, annealing at 60°C, and extension at 72°C for 60 s each in the presence of $MgCl_2$ at a final concentration of 2.25 mM.

PCR was conducted in a Perkin-Elmer Cetus GenAmp System 9600 (Perkin-Elmer, Norwalk, CT). PCR products were run on 2% agarose gel by electrophoresis. The transformed astroglioma cell line U373 that constitutively expresses αB-crystallin was used as a source of reference mRNA.

To amplify αB-crystallin-encoding mRNA from various species, pan-αB-crystallin-specific primers were designed to match all known sequences. cDNA (3 μl) was added to 1 μl 10 mM dNTP mix, 5 μl 10× *Taq* polymerase buffer (500 mM KCl, 600 μg · ml^{-1} BSA, 100 mM Tris-HCl (pH 8), 30 mM $MgCl_2$), and 1 unit *Taq* DNA polymerase (Life Technologies) in a final volume of 50 μl.

Pan-αB-crystallin primers were 5′-TGCRGTGACAGCAGGCTTCT-3′ and 5′-GAGAGCACCTGTTGGAGTCT-3′. Reaction conditions included premelting at 94°C for 4 min, followed by 40 cycles of melting at 94°C, annealing at 65°C, and extension at 72°C for 30 s each.

Western blot analysis

Whole cell lysates derived from EBV-LCL or the corresponding original PBMC were prepared by lysis in 20% (v/v) acetic acid supplied with 8 M urea. Standard SDS-PAGE analysis was done using an 8–25% gradient polyacrylamide gel (Pharmacia Biotech, Piscataway, NJ). For Western blotting, a 1/25 dilution was used of polyclonal rabbit Abs against human αB-crystallin that was isolated from MS-affected brains (16).

Viral infection of CBMC

CBMC were cultured in RPMI 1640 supplemented with 5% (v/v) pooled human serum at 10^6 ml^{-1} in a final volume of 10 ml in 25-cm^2 culture flasks. The cells were incubated for 48 h with 2.5 ml marmoset B95-8 cells culture supernatant containing EBV, 25×10^3 ml^{-1} Tissue Culture Infectious $Dose_{50}$ HHV-6 (strain U110Z) (33), 5×10^5 plaque-forming units per ml MV (strain Edmonston) (34), 33 hemagglutinating units per ml influenza virus A/HK/68, or 10 μg · ml^{-1} LPS (*Escherichia coli* 0127:B8; Difco, Brunschwig Chemie, The Netherlands).

Results

HLA-DR-restricted αB-crystallin-specific T cells proliferate in response to autologous EBV-LCL in the absence of exogenous Ag

Panels of TCL were generated from PBMC by weekly stimulation with purified αB-crystallin in the presence of irradiated, autologous PBMC as APC. As a rule, αB-crystallin-specific TCL were $CD4^+CD8^-$, expressed a TCR-αβ, and were HLA-DR restricted in their response to αB-crystallin (representative data for selected TCL are given in Fig. 1, *A* and *B*). All TCL were of the Th1 phenotype in that they produced significant amounts of the proinflammatory cytokine IFN-γ in response to αB-crystallin and little, if any, IL-4 (Fig. 1*C*). To date, no qualitative differences were found between TCL derived from either MS patients or healthy control subjects.

Following a culturing period of at least 7 wk using autologous PBMC as APC, TCL were transferred to a culture system employing autologous EBV-LCL as APC. Whereas αB-crystallin-specific TCL showed the expected dose-dependent response to their target Ag in the presence of autologous PBMC, they responded vigorously to autologous EBV-LCL in the absence of any exogenously supplied Ag. Representative data for three TCL are shown in Fig. 2*A*. In some (e.g., line S5L5), but not in all cases, addition of exogenous αB-crystallin resulted in a further enhancement of proliferative responses in a dose-dependent manner. The proliferative response to EBV-LCL both in the absence or presence of exogenously added αB-crystallin was HLA-DR restricted in that no responses were observed to HLA-DR-mismatched allogeneic EBV-LCL (data for S5L5 given in Fig. 2*B*) and that responses to autologous EBV-LCL could be blocked by mAbs to HLA-DR, but not to HLA-DQ (data not shown).

EBV infection of human B cells induces endogenous αB-crystallin expression

One explanation for the above data would be HLA-DR-restricted presentation of endogenously produced αB-crystallin by EBV-LCL. Expression of αB-crystallin was examined by amplifying mRNA by the reverse-transcriptase PCR (RT-PCR) using human αB-crystallin-specific primers. In all cases, readily detectable levels of αB-crystallin-encoding mRNA were present in EBV-LCL, but not in populations of PBMC before infection and transformation. Fig. 3*A* shows representative data obtained for two different donors. In accordance with these data, Western blot analysis of whole cell lysates revealed detectable levels of αB-crystallin only in EBV-transformed cells and not in untreated PBMC from the same donors (Fig. 3*B*). Finally, αB-crystallin expression in EBV-LCL was confirmed by immunocytochemistry, which revealed cytosolic and nuclear expression of the protein, while no such staining could be detected in unmanipulated PBMC (data not shown). The above findings indicate that upon infection and transformation by EBV, peripheral blood B cells start to synthesize αB-crystallin, leading to its presentation by HLA-DR molecules.

αB-crystallin expression in human lymphocytes results from viral infection rather than from transformation

Next, we examined whether or not endogenous expression of αB-crystallin in human lymphocytes is a unique consequence of EBV infection or whether perhaps other viruses or stimuli could have a similar effect. Human umbilical CBMC, free of prior viral infection, were exposed to a variety of stimuli, including infectious viruses. Expression of αB-crystallin-encoding mRNA was determined by RT-PCR following a 48-h culture period. Fig. 4 shows that αB-crystallin-encoding mRNA cannot be detected in unmanipulated cord blood cells nor in cells cultured in the absence of any stimulus. In vitro infection of cord blood cells with EBV, MV, HHV-6, or, to a lesser extent, influenza A virus triggered the production of detectable levels

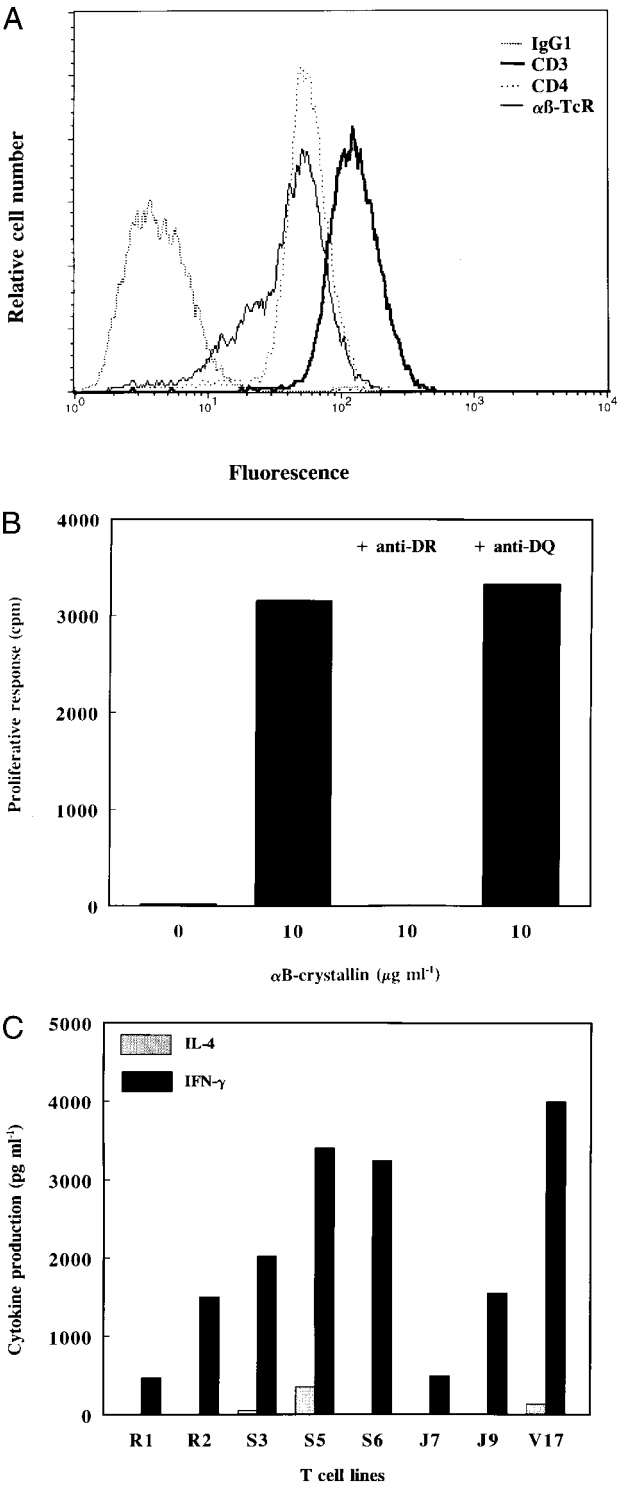

FIGURE 1. Characterization of αB-crystallin-specific human TCL. αB-Crystallin-specific TCL were characterized with respect to their expression of CD3, CD4, CD8, αβ-TCR and γδ-TCR, and HLA-DR-restriction and cytokine profiles. *A*, Expression of CD3, CD4, and αβ-TCR by the αB-crystallin-specific TCL S5L5. All TCL examined showed similar profiles, including negligible expression of CD8 or γδ-TCR (not shown). *B*, Anti-HLA-DR Abs abrogate Ag-specific responses by TCL S5L5. All TCL examined were similarly restricted to HLA-DR. *C*, Production of IFN-γ and IL-4 in response to αB-crystallin by a representative panel of specific TCL derived from four different donors.

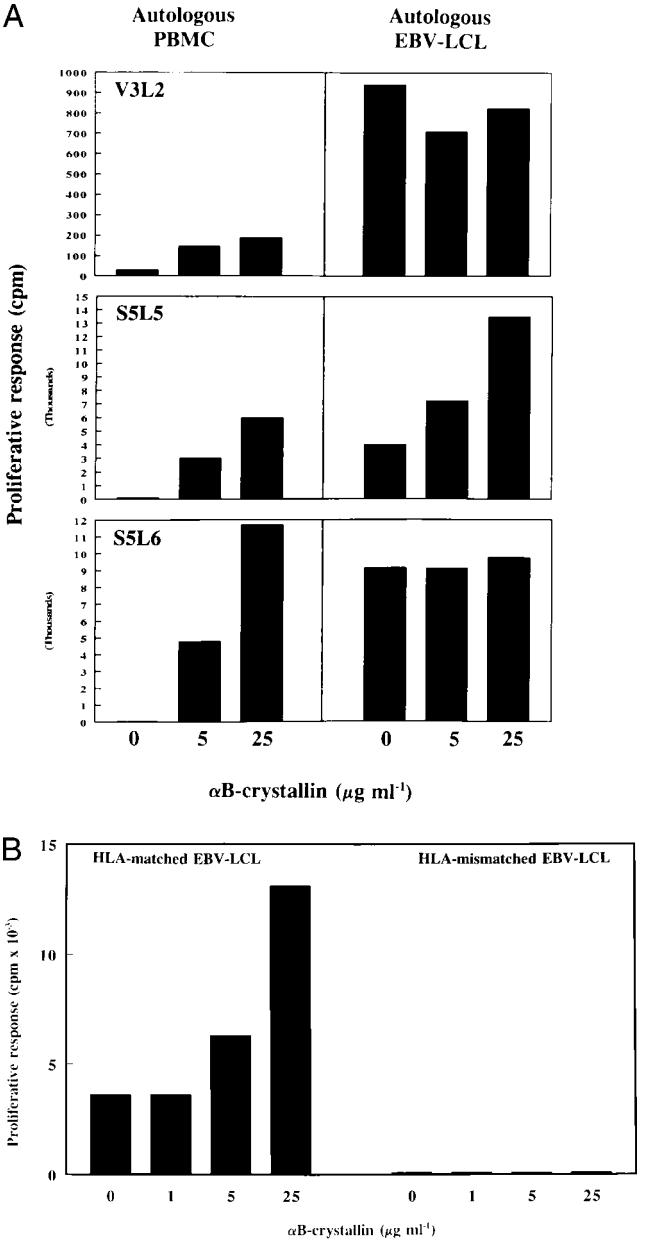

FIGURE 2. EBV-LCL activate αB-crystallin-specific TCL in the absence of exogenous Ag. *A*, αB-crystallin-specific TCL do not respond to PBMC without their Ag (*left-hand panels*), but do respond to autologous EBV-BCL in the absence of any exogenous Ag (*right-hand panels*). *B*, Responses to EBV-LCL in the absence as well as in the presence of exogenous αB-crystallin are HLA-DR restricted, as exemplified by the lack of responsiveness of TCL S5L5 to HLA-DR-mismatched allogeneic EBV-LCL.

of αB-crystallin-specific mRNA within a 48-h period. B cell-stimulating LPSs failed to produce such an effect.

To determine whether αB-crystallin expression in EBV-LCL could perhaps be associated with B cell transformation rather than with virus-associated events, we examined a panel of 10 EBV-negative human B cell lymphomas derived from patients with monoclonal gammopathies for the presence of αB-crystallin-encoding mRNA. In 5 of 10 EBV-negative B cell lymphomas, only trace levels of αB-crystallin-encoding mRNA could be detected at levels much lower than what can routinely be found in

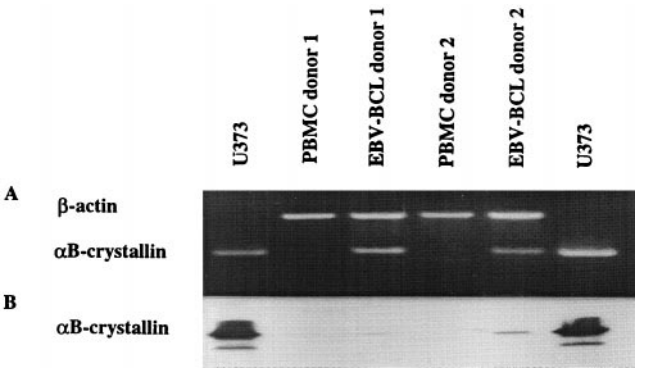

FIGURE 3. Upon infection and transformation with EBV, human B cells start to express αB-crystallin. Upon EBV transformation, peripheral blood B cells start to produce αB-crystallin-encoding mRNA and full-length protein, as visualized by RT-PCR (*A*) and Western blotting of whole cell lysates (*B*), respectively. No αB-crystallin can be detected in the PBMC from which the EBV-LCL were derived. Representative data are shown for two different donors.

EBV-LCL. In the other five lymphomas, no specific message was detectable at all (data not shown). Apparently, accumulation of αB-crystallin in lymphocytes is not uniquely associated with EBV infection, but still is an event of some selectivity and associated with viral infection rather than with mere transformation of B cells.

αB-Crystallin expression in peripheral lymphoid tissues is different from other hsp and varies among mammalian species

While the data in Figs. 3 and 4 already indicate that αB-crystallin is absent from healthy human PBL, the expression of αB-crystallin in PBMC was further examined in a larger panel of subjects and compared with other stress proteins by RT-PCR analysis. For this comparison, we focused on hsp27 as another small stress protein and on hsp60 as a representative of large stress proteins. The data consistently showed that αB-crystallin-encoding mRNA is absent from human PBMC, while readily detectable levels of mRNA-encoding hsp27 and hsp60 were found in all samples. Fig. 5 gives the results for a panel of six healthy donors.

These data prompted us to extend the analysis of αB-crystallin expression to other lymphoid tissues that are relevant to the induction and maintenance of immunologic tolerance, i.e., thymus and spleen. At the same time, we compared the expression of αB-crystallin in human tissues with corresponding tissues from several other mammalian species. For this purpose, PBMC and, when possible, spleen and thymus were collected from various strains of mice and rats, and sheep and primates, including chimpanzees, rhesus monkeys, marmosets, aotus, cynomolgous monkeys, and

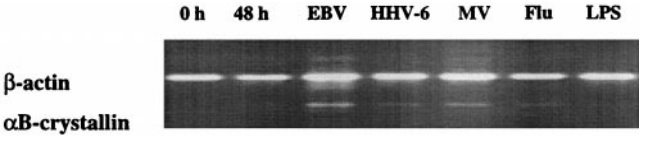

FIGURE 4. Virus-induced expression of αB-crystallin in human lymphocytes. RT-PCR analysis of αB-crystallin-encoding mRNA in CBMC following a variety of stimuli. Before RT-PCR analysis, CBMC were cultured for 48 h in the presence of EBV, HHV-6, MV, influenza A virus, or LPSs. For comparison, RT-PCR was also performed on EBV-LCL. As negative controls, unmanipulated CBMC and CBMC cultured for 48 h without any stimulus are shown. The transformed astroglioma cell line U373 that constitutively expresses αB-crystallin was used as a source of reference mRNA. Also shown is amplification of β-actin-encoding mRNA from all samples to verify adequate cDNA synthesis.

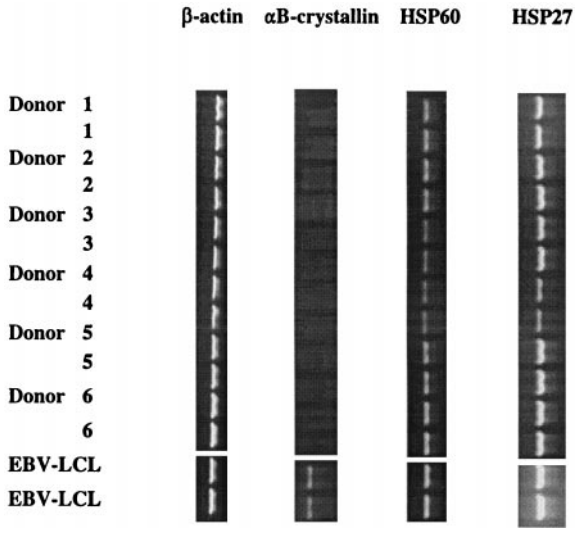

FIGURE 5. Constitutive expression of hsp27 and hsp60, but not of αB-crystallin in healthy human PBMC. RT-PCR comparison of mRNA levels encoding αB-crystallin, hsp27, and hsp60 in PBMC of healthy donors. Amplification of β-actin is shown as a control for adequate cDNA synthesis. Figure shows that hsp27 and hsp60 are expressed constitutively in all samples, but that αB-crystallin cannot be detected in any of them.

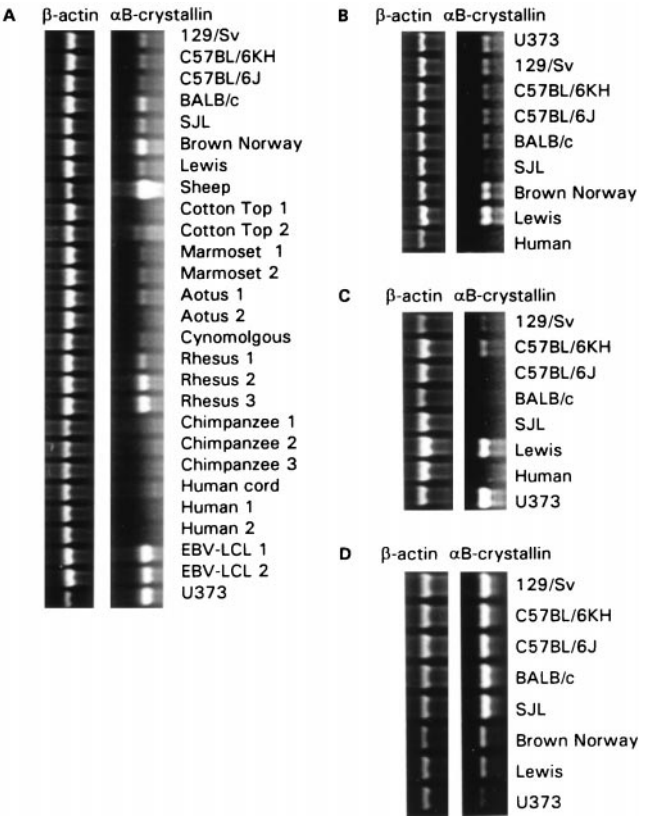

FIGURE 6. Expression of αB-crystallin in various mammalian species. RT-PCR was performed on mRNA isolated from PBMC (*A*), spleen (*B*), thymus (*C*), and, as a positive control, cardiac muscle (*D*) from various mammalian species using pan-αB-crystallin-specific primers. While αB-crystallin is expressed constitutively in PBMC and splenocytes from healthy rodents, sheep, and most primates, it is absent from human, chimpanzee, and cotton-top tamarin PBMC and from human spleen and thymus. Constitutive thymic expression of αB-crystallin was observed for 129/Sv and C57BL/6KH mice, for Lewis rats, and, in trace amounts, for BALB/c mice.

cotton-top tamarins. To deal with the (limited) sequence differences in αB-crystallin-encoding mRNAs in these different species, a set of pan-αB-crystallin primers was designed that match all known sequences. As a positive control, cardiac muscle from as many different species as possible was taken as a source of constitutively expressed species-specific mRNA. The human astroglioma cell line U373 that constitutively expresses αB-crystallin was used as a positive control for the amplification of human αB-crystallin-encoding mRNA.

The results of these analyses, shown in Fig. 6, clearly illustrate that in contrast to the absence of expression in human secondary lymphoid tissues, αB-crystallin-encoding mRNA is readily detectable both in PBMC and in spleen cells of other mammals (Fig. 6, *A* and *B*). This holds for all rodents examined and for most primates as well. As an exception, αB-crystallin-encoding mRNA was undetectable in SJL spleen despite expression in PBMC. At present, we have no explanation for this apparent discrepancy. Amplifications using pan-αB-crystallin primers failed to produce amplicons from chimpanzee as well as from cotton-top tamarin PBMC. When the amplification was repeated with the set of human-specific primers, cotton-top tamarin and chimpanzee PBMC remained negative (data not shown). When cardiac muscle was available as a control, amplification of species-specific mRNAs using the pan-αB-crystallin primers was always positive (Fig. 6*D*). In the case of chimpanzees and cotton-top tamarins, no cardiac muscle was available as a positive control nor information on the sequence of αB-crystallin in these species. Therefore, the possibility that in these two cases no specific message could be amplified from PBMC as the result of inadequate primer matching cannot be excluded.

The data in Fig. 6*C* further show that expression of αB-crystallin in the thymus is more restricted. Clearly, detectable levels of αB-crystallin were found in the thymus of only 129/Sv and C57BL/6KH mice and Lewis rats, while trace amounts were detected in the BALB/c sample. All other samples analyzed were negative.

Discussion

The present study documents some remarkable immunologic features of the small stress protein αB-crystallin. Contrary to other major stress proteins, αB-crystallin is not expressed constitutively in human secondary or primary lymphoid tissues, including PBMC, spleen, and thymus. This lack of expression is a property that humans may only share with chimpanzees and cotton-top tamarins, but absence of expression in PBMC of these latter species could not be formally proven due to the lack of positive control tissue. All other mammals examined displayed readily detectable constitutive levels of αB-crystallin in secondary lymphoid tissues, and in some cases even in the thymus.

Most likely, constitutive expression of αB-crystallin in secondary (and in some cases primary) lymphoid organs in most mammals results in a state of natural peripheral (and in some cases central) tolerance for the protein. In agreement with this notion, earlier reports have documented murine T cell tolerance for eye lens crystallins, including αB-crystallin (35, 36). Recent data confirm that thymic expression of autoantigens in rodents results in resistance to experimental autoimmune disease as can otherwise be induced by these Ags (37). Conversely, the lack of expression of αB-crystallin in human lymphoid cells may render the human immune system not tolerant to the protein. Not only would this condition of humans be unique as compared with other mammals, it

would also be quite different from the state of functional tolerance that must exist for other stress proteins, including hsp27, hsp60, hsp70, and hsp90, since these are expressed constitutively at appreciable levels in all human lymphoid tissues.

Against the above background, the appearance of αB-crystallin in virus-infected human B cells and the resulting presentation of the protein via HLA-DR to T cells may have interesting consequences in humans. Different from what would happen with other stress proteins and different from what would happen in most other mammals, generation of a T cell response to αB-crystallin is the likely result. All αB-crystallin-specific T cells that we have isolated to date from either MS patients or healthy control subjects are classical $CD4^+$Th1 cells that bear the αβ-TCR and produce IFN-γ and little, if any, IL-4 in response to Ag. At least in vitro, EBV, MV, HHV-6, and, to a lesser extent, influenza A virus appear to be able to switch on expression of αB-crystallin in human lymphocytes. Our data suggest that EBV is particularly effective in this respect.

As was shown previously, αB-crystallin is a dominant CNS myelin Ag for human Th cells in the CNS of MS patients (15). In addition, recent data show that subdominant or cryptic epitope of αB-crystallin is capable of inducing experimental allergic encephalomyelitis in mice (N.-M. Thoua, J. M. Van Noort, M. M. Morris, D. Baker, A. Bose, A. C. Van Sechel, M. J. B. Van Stipdonk, P. J. Travers, and S. Amor, in preparation). Thus, a virus-generated memory T cell repertoire specific for αB-crystallin that primarily involves Th1 cells is cross-reactive with CNS myelin and should be considered as potentially pathogenic. When such a T cell repertoire would be recruited into the CNS under conditions that also lead to the local accumulation and presentation of αB-crystallin, a pathogenic inflammatory burst could well be the result. Yet, each of the contributing factors, including the presence of a virus-induced peripheral repertoire against αB-crystallin, would not necessarily be pathogenic on their own. Recent data show that recruitment of lymphocytes and accumulation of αB-crystallin in the oligodendrocyte/myelin are precisely what can be observed in early active stages of lesional development in MS, while they are not observed in inactive plaque areas or healthy control tissue (16).

It is tempting to relate the presently observed capacity of EBV to trigger presentation of αB-crystallin in secondary lymphoid cells to the existing epidemiologic evidence implying EBV infection as a risk factor in the development of MS (11, 13, 14). Several studies have shown that 99–100% of MS patients are EBV seropositive (38, 39), consistent with EBV infection being a necessary (but by no means sufficient) contributing factor. In addition, evidence has been reported to indicate that an episode of infectious mononucleosis, which is characterized by an exaggerated T cell response to EBV-infected B cells, significantly increases the risk to develop MS later on in life (11, 12, 14, 40, 41). While evidence for an involvement of EBV in the development of MS remains only circumstantial, data such as the above cannot be fully ignored. Our present study provides a novel mechanism by which the contributions to MS of relatively common viral infections on one hand and CNS myelin-directed T cell responses on the other may be reconciled.

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