Increased Apoptosis of CD20+ IgA+ B Cells is the Basis for IgA Deficiency: The Molecular Mechanism for Correction In Vitro by IL-10 and CD40L

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Received August 2, 2005; accepted October 24, 2005
Published online: 26 April 2006

IgA deficiency is the most common primary immunodeficiency in humans. Comparative analysis of gene expression in PBMC from IgA-deficient (IgAd) and normal donors using functional multiplex panels showed overexpression of the Caspase-1 (CASP-1) gene. Cells from all the IgAd donors (n = 7) expressed 4–10-fold caspase-1 mRNA over normal controls (n = 5). CD19+ B cells from all IgAd donors produced IgA in cultures following IL-10 and CD40L with Staphylococcus aureus (Cowan) (SAC) or tetanus toxoid (TT) treatments. In CD19+ B cells from IgAd donors, reconstitution of IgA secretion was associated with protection of the CD20+ B cell population that underwent apoptosis in the absence of IL-10, CD40L, and TT (triple treatment). Caspase-1 gene expression was decreased in the reconstituted cells. Furthermore, treatment with a caspase-1 inhibitor also independently protected against B cell apoptosis in vitro. An apoptosis-specific cDNA array showed differential expression of 4 out of 96 genes and a shift towards survival-related gene expression from the apoptotic to the protected B cells after triple treatment. There was an increase in the expression of the IAP-2 (inhibitor of apoptosis) gene in the reconstituted cells. Upregulation of the IAP-2 gene protects B cells from deletion and allows for IgA secretion in this system. The inability to detect secreted IgA in IgAd patients could result from the loss of IgA-committed B cells that express high levels of caspase-1.

KEY WORDS: Immunodeficiency diseases; IgA deficiency; caspase-1; apoptosis.

INTRODUCTION

IgA deficiency (IgAd), first described in Henry Kunkel’s laboratory at Rockefeller University in 1964 (1), is the most common form of primary immunodeficiency and affects one in 400–3000 individuals (2). Prevalence of the disease varies across ethnic groups and is higher among Caucasians (1/600) than among Japanese (1/18,000) (3–5). Although lack of IgA does not often cause severe clinical symptoms, secretory IgA protects against a number of foreign antigens and pathogens. IgAd is associated with a wide variety of disorders including allergies, respiratory tract infections, autoimmune diseases (rheumatoid arthritis, ITP, hemolytic anemia, systemic lupus erythematosus), gastrointestinal diseases and common variable immunodeficiency (3–5). The disease may be heterogeneous and patients include individuals with inherited, drug-induced, transient forms or other Ig class or subclass deficiencies. Patients with a compensatory increase in secretory IgM are less symptomatic (6) while those with other subclass deficiencies have more severe sinopulmonary infections (7).

Unlike monogenic immunodeficiency diseases, IgAd inheritance is quite complex. Patients’ family members (8) are often unaffected and a monozygotic twin of an IgAd patient can have normal IgA levels (9) (Z. Husain et al., unpublished results). This suggests that a susceptibility gene for IgAd is incompletely penetrant. Several studies have shown an increased frequency of certain conserved extended MHC haplotypes such as [HLA-B8, SC01, DR3], [HLA-B57, SC61, DR7] (10), [HLA-B44, FC31, DR7] (11) and [HLA-B65, SC2(1,2), DR1] (12) in IgAd patients. Homozygotes for [HLA-B8, SC01, DR3] have a markedly increased frequency (~13%) of IgAd whereas heterozygotes do not (13), indicating recessive inheritance of a susceptibility gene on this haplotype.
Susceptibility genes may lie in the MHC class II region (14), or perhaps near the TNF region (3) or these and/or a second class II region near HLA-DRBI (15).

While the molecular basis for IgAd is not yet known, a failure of isotype switching, or of terminal differentiation of B cells, or premature death of precursors of IgA-producing B cells could lead to IgAd. The IgA structural gene is normal in IgAd (16). A defect in $\gamma$ to $\delta$ recombination associated with decreased levels of $\alpha$ membrane mRNA has been reported in a subset of IgAd patients (17). We found that, although all IgAd patients had markedly reduced transcription of the membrane and secreted forms of IgA, patients had a spectrum of severely defective to normal switching from IgM to IgA (18).

Brière and colleagues have shown that IgAd B cells can be induced to secrete IgA in vitro by IL-10, CD40L and SAC (“triple treatment”) (19). This could be mediated either by eliminating the block in differentiation or by a reversal of death of precursors. Our results suggest that B cells destined to produce IgA undergo apoptosis by a previously unrecognized pathway involving caspase-1, a cysteine protease involved in the processing of IL-1β and IL-18. Protection from this form of apoptosis is afforded by IL-10 and CD40L and potentiated by SAC or TT (the latter as substitute for SAC).

MATERIALS AND METHODS

Human Subjects

Seven IgAd patients whose serum IgA levels were 5 mg/dL (measured by IgA ELISA, see later) or lower were used in the present study. Three were members of the same family. Control subjects ($n = 5$) were selected from healthy, random blood donors who had normal Ig levels. Four of the donors were male and three were female. Patients ranged in age from 20 to 74 years. All patients and control donors signed informed consent forms approved by the Institute’s IRB.

Reagents

The caspase-1 inhibitor Ac-YVAD-CHO was obtained from Alexis Biochemicals (San Diego, CA). Formalinized particles of S. aureus strain Cowan I (SAC) were obtained from Calbiochem (La Jolla, CA). Tetanus toxoid was obtained from Connaught Laboratories (Willowdale, ON). Antibody against caspase-1, sc-622 (rabbit IgG), was obtained from Santa Cruz Biototechnology (Santa Cruz, CA). CD20-FITC (2H7, mouse IgG₁), CD19-PE (HIB19, mouse IgG₁) and isotype control antibodies were obtained from BD Biosciences (San Diego, CA).

CD19+ B Cell Purification

PBMC were obtained from heparinized venous blood samples by Ficoll-Paque (Pharmacia, Piscataway, NJ) gradient centrifugation. Cells were either used immediately or frozen in a solution of 20% heat-inactivated pooled human serum (PHS) (Nabi, Miami, FL), 10% dimethylsulfoxide (DMSO), 1% penicillin-streptomycin and 1% L-glutamine (all from Cellgro, Fisherbiotech) in RPMI. PBMC were thawed if frozen and washed twice with a medium of 44% DMEM (BioWhittaker, Walkersville, MD), 44% F12 (Life Technologies, Rockville, MD), 10% heat-inactivated fetal calf serum (Atlanta Biologicals, Norcross, GA), 1% pen/strep, and 1% L-glutamine. Before CD19+ cell selection, nonspecific binding was minimized by resuspending cells in a solution of 10% human IgG (CBR Laboratories, Inc., Boston, MA) and 10% heat-inactivated PHS in AIM V media at 100 $\mu$L per 10 x 10^6 cells and incubating on ice with rocking for 30 min. After blocking for nonspecific binding, cells were washed with PBS, and MACS anti-CD19 beads (Miltenyi Biotec Inc., Auburn, CA) were added at 20 $\mu$L beads per 10 x 10^6 cells. Cells were incubated on ice for 30 min and sorted on a Miltenyi Automacs instrument. The CD19+ fraction was collected and counted with trypan blue. Yield of viable cells was generally 5–15% of total PBMC.

Culture Conditions

CD40L-expressing NIH 3T3 (CD40L+T) cells, kindly donated by Dr. Joachim Schultzze of the Dana-Farber Cancer Institute, Boston (20), were irradiated (6210 rad) before plating at 5 x 10^3 or 1 x 10^4/well in flat-bottom plates in 44% DMEM (BioWhittaker), 44% F12 (Gibco BRL), 10% heat-inactivated fetal calf serum, 1% pen/strep, 1% L-glutamine, and geneticin (Gibco BRL) at 400 $\mu$g/mL. CD 19+ B cells were added at 5 x 10^4/well and IL-10, SAC (or TT) were added to a final concentration of 100 ng/mL, 0.005%, and 2 $\mu$g/mL, respectively. Plates were incubated at 37°C in 5% CO₂. EBV-transformed B cells used in ELISA and RT-PCR experiments were seeded at 2 x 10^6 cells in 2 mL of RPMI culture media and FCS and have been described previously (21).

Cell Staining and Measurement of Apoptosis

B cells (1.5 x 10^6) were used for staining with anti-CD20-FITC and annexin V-FITC or annexin V-biotin, and for terminal dUTP nick-end labeling (TUNEL) assay for measurement of apoptosis. Cells stained with CD20-FITC...
and annexin V-biotin were first washed in 1 × binding buffer. After washing, CD20-FITC and annexin V-biotin were added to the cells resuspended in 100 μL of 1 × binding buffer. Cells were then incubated for 30 min at 4°C in the dark and resuspended in 100 μL of 1 × binding buffer following washing. Streptavidin-PE was then added to the cells which were incubated at 4°C for 30 min. Cells were then washed three times and resuspended in 200 μL of 1 × binding buffer and analyzed on a Becton-Dickinson FACSCalibur using Cell Quest software. Cells stained with the ApoAlert Annexin V-FITC Apoptosis kit (Clontech, Palo Alto, CA) were washed three times in 1 × binding buffer and resuspended in 200 μL of 1 × binding buffer. Cells were incubated at room temperature in the dark following addition of 5 μl annexin-V. The TUNEL assay was performed using the MEBSTAIN Apoptosis Kit II (MBL, Nagoya, Japan) following the manufacturer’s suggested protocol.

IgA ELISA

Nunc Immuno-Polsorp plates (Naperville, IL) were coated with rabbit anti-human IgA (Sigma) in 0.1 M NaHCO₃. After incubation overnight at 4°C, plates were washed with PBS/0.05% Tween20. After washing, plates were incubated with PBS/1.0% BSA at 100 μL/well for 1 h at 37°C to block nonspecific activity. Serum samples or cell-free supernatants were then diluted in PBS/0.1% BSA and incubated at 37°C for 2 h. Following washing, goat anti-human IgA alkaline phosphatase-conjugated antibody (Pierce, Rockford, IL), diluted 1:5000 in PBS/0.1% BSA, was added. The plates were incubated for 1 h at 37°C. After a final wash, a substrate of p-nitrophenylphosphate dissolved in diethanolamine was added. The plates were then incubated at 37°C for 1 h and optical densities were measured at 405 nm on a Dynatech MRX plate reader. Calculations were made from the standard curve generated with known concentrations of purified human IgA (Sigma).

mRNA Extraction, Single Gene and Multiplex PCR

RNA was isolated using the Ultraspec RNA reagent (Biotecx Laboratories, Houston, TX) and cDNA was synthesized with M-MLV reverse transcriptase. PCR was performed using Taq polymerase (2.5 U), MgCl₂ (1.5 mM), and dNTP (0.4 mM) with cycling conditions as follows for GAPDH and CASP-1: 94°C for 30 s, 58°C for 30 s, 72°C for 1 min with a final extension at 72°C for 7 min. A commercially available multiplex PCR (MPCR) kit of TNF-signaling genes (Maxim Biotech, So. San Francisco, CA) was also used. The following conditions were used: two cycles of 96°C for 1 min and 58°C for 4 min; 30 cycles of 94°C for 1 min and 58°C for 2.5 min, followed by extension at 70°C for 10 min and soak at 25°C. The following primer sets were used: GAPDH, 5’-CGACCACCTTTGTCAGCTCA-3’ and 5’-AGGCTTCAATGGCAGG-3’; CASP-1, 5’-GGATGTCAAGCTTGTCG-3’ and 5’-TGCTGT-CAGAGGCTTTGTC-3’; and TNF-α, 5’-ATGAC-GGATCTGAGCTCGG-3’ and 5’-GCAATGATCCCGATCTGC-3’. PCR products were visualized under UV in 2% ethidium bromide-stained agarose gels. In some cases, PCR-amplified fragments were analyzed on agarose gels followed by scanning and quantitation using an IS-1000 digital imaging system (Alpha Imnotech Corporation, San Leandro, CA).

Caspase-1 Gene Expression and Quantification by Real-Time Quantitative RT-PCR

RNA was isolated from CD19⁺ B cells, cDNA was synthesized and gene expression was quantified using the Taqman PCR Reagent Kit (Applied Biosystems, Foster City, CA) and the iCycler (Bio-RAD, Hercules, CA). The following forward, reverse primers and Taqman probes were designed for GAPDH and CASP-1 respectively: 5’-TGTCCAAATATGGTCAAC-3’, 5’-GGATTCCCATTGAGCAAG-3’, FAM-CACCGT-CAAGGCTAGAACG-TAMRA; 5’-AGCTTGTGTTCATCCTGTA-3’, 5’-TGCCAGGTAACGTTGTCCTT-3’, FAM-CAGGCTGACCAATTTGCT-TAMRA. GAPDH was used to normalize differences in amounts of RNA among samples.

Quantitation of Caspase-1 Protein

Twenty to forty micrograms of whole cell extracts were subjected to 10% SDS/PAGE and the resolved proteins were transferred electrophoretically to PVDF membranes and blotted for the presence of human caspase-1 protein using the anti-caspase-1 antibody sc-622 (Santa Cruz Biotechnology). Chemiluminescence detection was performed using the ECL system (Amersham, Piscataway, NJ) according to the manufacturer’s instructions.

Functional Microarray of Apoptosis-Related Genes

Human Apoptosis GEArray Q Series (SuperArray Inc., Bethesda, MD) microarrays were used to analyze expression of survival and death-related genes. CD19⁺ B cells were cultured for 72 h in the presence or absence of IL-10, CD40L, and TNF. Cells were harvested, mRNA was extracted and biotinylated dUTP cDNA probes were synthesized. Relative gene expression analysis was performed by visual inspection. Microarray
results were validated by gene-specific RT-PCR using
cDNA isolated from untreated and triple-treated cells.
The forward and reverse primers used were, respectively: LIGHT: 5′-GTACGCGCTCTAGTTTTGT-3′;
LIGHT: 5′-GAAAGCCCCGAAGTAAAGACC-3′; IAP2: 5′-ATTTGATGAAAAGCAGCAAC-3′; IAP-2: 5′-AAG-
CAAGCACTCTGTCTCC-3′.

RESULTS

**IgA Production in B Cells from IgAd Donors**

IgAd B cells (phenotypically similar to normal B cells
by the markers studied, Table I) secreted no detectable
IgA on culture for 8 days, whereas B cells from normal
donors did (Fig. 1 and data not shown). However, B cells
from IgAd patients secreted significant levels of IgA in
culture in the presence of CD40L, IL-10 and SAC, as
reported earlier (19). We also found TT to be as effective
as SAC as a potentiator of this effect. IL-10 and CD40L
(with TT or SAC) also enhanced IgA secretion in cultured
B cells from normal donors. Individual treatments by IL-
10, antigen (SAC/TT) or CD40L did not produce any
significant increase in IgA secretion (Fig. 1 and results
not shown).

**Multiplexed Analysis of TNF Signal-Related Genes**

Since our preliminary gene mapping studies had sug-
gested that the TNF-region was one of the candidate re-
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were no differences between IgAd patients and healthy controls for the expression level of any of the other genes in the panel.

**Caspase-1 and TNF-α Gene Expression**

To validate the results obtained with multiplex PCR, we carried out single-gene RT-PCR with RNA isolated from PBMC and B cells. Caspase-1 gene expression was upregulated in B cells in all the IgAd patients studied compared with normal donors (Fig. 2B). Densitometric scanning of gels showed increases of five- to ninefold in patients compared to controls after normalization against internal GAPDH expression levels (Fig. 2C). However, in both PBMC (data not shown) as well as in B cells, there was no difference between IgAd and normal subjects in the expression pattern of TNF-α (Fig. 2D) or the other genes in the multiplex panel (data not shown).

**Quantitation of Secreted Caspase-1, IL-1β, and IL-18**

We also found overexpression of the caspase-1 gene and its protein product in IgAd B cells by western blot analysis and real-time quantitative RT-PCR assay (Fig. 3). Quantitative analysis of gene expression showed an increase similar to that observed after RT-PCR (about a sevenfold increase in caspase-1 expression in the IgAd B cells) (Fig. 3B). Further analysis of caspase-1 protein in the cell lysates from normal and IgAd donor B cells showed the precursor p45 kD protein in both but significant levels of the active form of the cleaved p20 form were present only in the patients’ B cells (Fig. 3A).
and 30% [48 h]) as compared with those obtained from normal donors (70% [24 h] and 67% [48 h]) (Fig. 4A). Furthermore, when annexin-positivity was calculated as a percentage of the total remaining CD20+B cells in culture, the percent annexin-positive cells was also consistently higher in the cells obtained from the IgAd donors (52% [24 h] and 89% [48 h]) as compared with those obtained from normal donors (32% [24 h] and 42% [48 h]) (Fig. 4B). A TUNEL assay showed a similar pattern of CD19+B cell apoptosis in culture over a 48 h period (Fig. 4C). Thus, TUNEL-positive IgAd donors’ B cells ranged from 58% (24 h) to 70% (48 h) compared to 21% (24 h) to 42% (48 h) for normal donors’ B cells. A similar pattern of B cell apoptosis was observed when CD19+B cells were isolated by negative selection from the same IgAd and normal donors (results not shown).

IgA Reconstitution is Associated with Protection Against B Cell Apoptosis and Downregulation of Caspase-1 mRNA

Since B cells from IgAd patients produce IgA in the presence of IL-10, CD40L and TT, we analyzed the differential survival of these cells as compared with untreated cells in culture. Annexin-positive untreated IgAd cells comprised 73% of gated cells after 5 days in culture, but this percentage decreased to 34% in the treated population (results not shown). While about 6% of cells in the untreated population were CD20+, triple treatment resulted in 45% of the cells staining for CD20, suggesting that a significant portion of IgAd CD19+B cells became CD20+ cells and survived in culture to produce IgA (Fig. 5A). While similar protection against apoptosis was also seen in the normal donors’ B cells, the major difference between the initial and final populations of the total CD20+ population was clearly evident. Untreated IgAd CD19+B cells placed in culture for 5 days retained high levels of caspase-1 gene expression (Fig. 5B). However, cells receiving treatment with IL-10, CD40L and TT had a decreased level of caspase-1 mRNA when compared with controls (about threefold lower by densitometric analysis).

Decreased Apoptosis of CD19+B Cells Following Cell-Permeable Caspase-1 Inhibitor (Ac-YVAD-CHO) Treatment

We treated CD19+B cells from IgAd and normal donors with Ac-YVAD-CHO for 24 h and analyzed apoptosis in these cells. Figure 6A shows that significant apoptosis (50.1%) was detected in IgAd donors’ B cells compared to normal donors’ cells (18.9%). However, after 24 h of inhibitor treatment, the percentage of annexin-positive

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**Fig. 3** Caspase-1 levels in normal and IgAd donors’ freshly isolated B cells. (A) Expression of caspase-1 protein in IgAd and normal CD19+B cells by Western blotting. The IgAd donor’s B cells expressed both inactive (45 kD) and the active forms of cleaved 20 and 10 kD fragments. (B) Caspase-1 gene expression in CD19+B cells from normal and IgAd donors determined by quantitative real-time RT-PCR. RNA isolated from freshly isolated B cells was subjected to real-time RT-PCR and Ct values were used to calculate relative expression levels. All the examples shown are representative of three identical experiments.

**Increased CD20+B Cell Apoptosis in IgAd**

We used CD20 and annexin positivity to assess the survival potential of CD19+B cells in culture. The total CD20+B cells remaining in culture was consistently lower in the cells obtained from IgAd donors (53% [24 h]
cells decreased to 28.7% in the IgAd donor B cells. There was also a slight decrease in the percentage of annexin-positive B cells from normal donors (10.8%). The TUNEL assay further substantiated our observation of apoptotic cell death by demonstrating about 58% apoptotic cells in IgAd donors’ B cells after 24 h in culture (Fig. 6B). The presence of the caspase-1 inhibitor Ac-YVAD-CHO decreased the percentage of apoptotic cells to 26.9%.

Functional Microarray of Apoptosis-Related Genes

Analysis of an array comprising 96 apoptosis-related genes was carried out using CD19+ B cells from an IgAd patient following 72 h culture with or without the triple treatment. Seventeen of these genes were expressed in these cells, with four genes showing differential expression between treated and untreated cells (Fig. 7A). Three genes were expressed at higher levels in untreated cells (compared to treated cells). These were TRAF1, BAK1 and LIGHT. The only gene that was upregulated in the triple treated cells was IAP-2, a member of the “inhibitor of apoptosis” gene family. The most striking differences in expression pattern were for LIGHT (highly expressed in untreated cells, weakly expressed in untreated normal B cells) and IAP-2, which showed very low expression in the untreated cells but was abundantly expressed in treated cells. Confirmation of the microarray data was carried out by single-gene RT-PCR analysis in independent reconstitution experiments (two separate IgAd donors) using LIGHT and IAP-2-specific primers (Fig. 7B). Other
caspases, including caspase-3, -7, -8 and -10, and survival factors including Bcl-2 and Bcl-X showed no differences in expression (data not shown).

**DISCUSSION**

In this study, we have shown that IgAd is associated with a loss of B cells *in vitro* through apoptosis. Our results show that IgA⁺CD20⁺ B cells are selectively eliminated via a previously undescribed pathway in B cells involving caspase-1. Moreover, protection of these cells by IL-10 and CD40L or the presence of a caspase-1 inhibitor downregulates caspase-1 mRNA and inhibits apoptosis associated with induction of IAP-2. We confirm earlier findings (19) of IgA production in IgAd patients’ PBMC and B cells in a system *in vitro* that included IL-10, CD40L and SAC. Our results elucidate the mechanism involved in the reconstitution of IgA production by IL-10 and CD40L by B cells from IgAd individuals. We observed that B cells from all the patients tested responded to the combination treatment mitigating against heterogeneous defects. Also, while the secreted IgA response levels varied from low response in some donors to increases that approached normal levels in others, the pattern of response (IL-10, CD40L, TT alone or in combination) essentially remained

![Fig. 5](https://example.com/fig5.png)  
**Fig. 5** Triple treatment results in B cell survival and decreased caspase-1 levels. (A) PBMC from an IgAd and a normal donor were positively selected for CD19⁺ cells and both were placed in culture in the presence or absence of IL-10, CD40L and TT. After 5 days, cells were stained with anti-CD20-FITC. Shown are the percentage of CD20⁺ cells, which are representative of two separate experiments using two different normal and IgAd donors. (B) Cells were harvested after 48 h from a similar experiment and used to determine caspase-1 expression by RT-PCR. Data shown are representative of one of three separate experiments.
Fig. 6 Caspase-1 inhibitor protects against B cell apoptosis. (A) CD19+ B cells from normal (n = 2) and IgAd donors (n = 2) were placed in culture for 24 h with and without the caspase-1 inhibitor Ac-YVAD-CHO. Cells were stained for annexin positivity to determine apoptosis. Protection against apoptosis was observed in caspase-1 inhibitor-treated cells. (B) TUNEL assay of CD19+ B cells similarly treated with caspase-1 inhibitor showing protection against cell death following Ac-YVAD-CHO treatment. Data shown representative of one of three separate experiments.

Thus, in all the patients studied, only the combination treatment with IL-10 and CD40L allowed for IgA reconstitution, while no single activator induced IgA secretion. TT or SAC merely potentiated the combined effects of IL-10 and CD40L. All IgAd-B cells had increased expression of the caspase-1 gene that ranged from 4–10-fold higher in patients compared to controls. This was observed first in a multiplex RT-PCR of TNF-signaling genes and was subsequently corroborated in single gene RT-PCR analysis.

Journal of Clinical Immunology, Vol. 26, No. 2, 2006
in B cells. No changes were observed in the expression of other genes studied, including TNF-α, NFκB, \( \text{IkB} \) an and \( \text{Bcl-2} \), as shown in Fig. 2, nor in the levels of caspase-3, caspase-9, IL-10, IL-12, IFN-γ nor IL-1β (results not shown). Parallelly increased caspase-1 mRNA, the levels of functionally active caspase-1 \( \text{p20/p10} \) protein levels in the B cells from IgAd patients were \( 8–10 \)-fold higher than in the control B cells. Our results support a role of caspase-1 as an upstream effector inducing apoptosis of B cells without the involvement of caspase-3. It is likely that caspase-1-mediated apoptosis involves a separate pathway that may bypass caspase-3 activation before activating specific endonucleases. Since it has been reported that PARP (poly ADP-ribose polymerase)-cleavage requires \( 30–50 \)-fold more caspase-1 than caspase-3 (22), potential roles of other substrates in this pathway are likely.

Caspase-1, one of the intracellular cysteine proteases first described as the IL-1β converting enzyme (ICE) (23), plays a role in several apoptotic pathways (24–26). It is present as a functionally inactive 45 kD precursor that requires two internal cleavages to become the functionally active \( \text{p20/p10} \) heterodimer (27). While the role of caspase-1 in lymphoid cell apoptosis is unclear, its participation in apoptosis has been shown in various other systems (28–31). In C. elegans, transfection of cells with caspase-1 induces apoptosis that is inhibited by co-transfecting with the inhibitor \( \text{cma} \) (32). In gastric cancer cells, cisplatin-induced apoptosis is associated with caspase-1 activity (33). Also, \( \text{Shigella} \) induces a form of apoptosis that is dependent on caspase-1, but does not require caspase-3 (34). The intranuclear Huntington protein (huntingtin) induces expression of caspase-1 that mediates apoptosis in neuronal cells (35). Thus, caspase-1 mediates early events in apoptosis that may (35, 36) or may not (34) involve downstream caspase-3 activity. Also, NO induces apoptosis in thymocytes via a mechanism involving a caspase-1 pathway (37) such that thymocytes in caspase-1 knockout mice, but not in caspase-3 knockout mice, undergo NO-mediated apoptosis that could be inhibited by the caspase-1-specific inhibitor Ac-YVAD-CHO. A recent report also observed delayed apoptosis of neutrophils in caspase-1 knockout mice and showed that this apoptosis could be reversed by caspase-1 inhibitor (38).

The role of IL-10 in B cells has been extensively investigated and implicated in B cell proliferation and survival (39). Both exogenous and B cell-derived IL-10 affects isotype switching and differentiation. IL-10 has been shown to be a switch factor for IgG1 and IgG3 (40, 41), and, together with TGF-β, for IgA (39, 42). However, no defects in IL-10 production were seen in patients with IgAd (39). Moreover, experiments involving IL-10−/−

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**Fig. 7** DNA microarray analysis of apoptosis and survival-related genes. (A) RNA isolated from CD19+ cells from an IgAd donor receiving triple treatment (3T) or no treatment (no treat) for 72 h was used to probe membranes to detect expression of 96 genes. Levels of expression denoted by hybridization intensity representing + (low), ++ (moderate) or +++ (high). (B) Validation of microarray data performed independently using single gene-specific primers and RT-PCR in CD19+ cells from normal (n = 2) and IgAd donors (n = 2). Results are shown for the two genes that showed the most striking differential expression (IAP-2 and LIGHT) between normal and IgAd donor cells either before or after treatment. These results are representative of three separate experiments (data presented is for one donor’s cDNA sample)
and IL-10 transgenic mice, gene delivery or anti-IL-10-neutralizing antibody-based blocking (43-45) have not suggested a major role in vivo for IL-10. But it is possible that IL-10 protects against B cell apoptosis via activation of the Bcl-2 gene, with this protection depending on the state of activation of the B cells (46, 47).

B cell development involves two phases: an antigen-independent pre-B cell stage in the bone marrow where an antigen-specific diverse repertoire of B cells develops, and a second phase in which B cells undergo clonal expansion in peripheral lymphoid tissue following antigen activation. Memory B cells from the peripheral blood differentiate into IgG-secreting cells following CD40 ligation and cytokine stimulation (48). In such a system, a population of apoptotic cells (up to 15% of apoptotic and 30% of dead cells) was observed after 8 days in culture, and it was suggested that lack of an essential signal following switching may result in elimination of the switched naïve peripheral B cells (48). Dadgostar et al. (49) also showed that spontaneous apoptosis of mouse B cells could be protected by CD40L. After 24 h in culture, over 70% of cells underwent apoptosis as measured by annexin-positive. Our results show that upregulation (or lack of downregulation) of an apoptotic signal causes elimination of B cells in culture, and that the presence of two signals mediated by CD40L and IL-10 are sufficient to abrogate this deletion process. Since it has been previously suggested that proliferation of B cells is necessary for generation of antibody-secreting cells (50, 51), it is quite likely that appropriate survival factor expression is crucial to this event. Although T cell function has been considered to be normal in IgAd (52), the ability to reconstitute IgG secretion with just two factors (CD40/CD40L interaction and IL-10) as well as the multitude of defects in T cells in other primary immunodeficiency diseases suggests that there could be a T cell dependent process that is related to the inability to generate IgA-producing B cells in these patients.

While the nature of events leading to B cell apoptosis is still unclear, the role of unknown caspases in early plasma membrane changes associated with B cell apoptosis has been suggested (53). However, no published report has shown the role of a specific caspase in B cell apoptosis. We present the first evidence of the role of caspase-1 in B cell apoptosis and demonstrate its association with IgAd. Our results also show the involvement of the anti-apoptotic gene IAP-2 in B cell survival. While the IAPs protect against apoptosis by binding and neutralizing various caspases (54), their possible role in B cell survival has not been previously well documented. Our results show for the first time that, in a reconstitution system in vitro, IAP-2 upregulation may be sufficient to protect B cells from apoptosis and no other survival factors are involved in this system.

Increased apoptosis of B cells following activation prevents IgA+ B cells from differentiating into IgA-secreting plasma cells in these patients. While the involvement of IAP2 in B cell survival seems evident, the potential role of LIGHT (a recently identified member of the TNF superfamily expressed in activated T cells and dendritic cells) remains unclear. Intriguingly, a recent paper (55) proposes a role for LIGHT in costimulation of B cells following early CD40L activation. Moreover, the authors also observed LIGHT activation at the transcriptional level in activated B cells. They suggest a “trans”-priming role of LIGHT in activated B cells. It is conceivable that aberrant transcription of LIGHT in IgAd B cells might have a negative effect on CD40L signaling or have a downstream effect on plasmocyte generation. IL-10 and CD40L in this system provide a balance of B cell survival and proliferation along with downregulation of endogenous caspase-1 transcription, thereby providing the requisite environment for survival and differentiation of IgA-producing cells. Thus, there is clear evidence that the inability to produce IgA in IgA deficiency is due to the increased destruction of a subset of B cells in these patients.

ACKNOWLEDGMENTS

We sincerely thank Drs Keith Crawford, Devendra Dubey, Charles Larsen and Judy Lieberman for their review of the manuscript and numerous discussions and suggestions. This work was supported by grant HL-29583 from the National Heart, Lung and Blood Institute of the National Institutes of Health.

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