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Targeting Positive Regulatory Domain I-Binding Factor 1 and X Box-Binding Protein 1 Transcription Factors by Multiple Myeloma-Reactive CTL

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Growing evidence indicates that multiple myeloma (MM) and other malignancies are susceptible to CTL-based immune interventions. We studied whether transcription factors inherently involved in the terminal differentiation of mature B lymphocytes into malignant and nonmalignant plasma cells provide MM-associated CTL epitopes. HLA-A*0201 (A2.1) transgenic mice were used to identify A2.1-presented peptide Ag derived from the plasma cell-associated transcriptional regulators, positive regulatory domain I-binding factor 1 (PRDI-BF1) and X box-binding protein 1 (XBP-1). A2.1-restricted CTL specific for PRDI-BF1 and XBP-1 epitopes efficiently killed a variety of MM targets. PRDI-BF1- and XBP-1-reactive CTL were able to recognize primary MM cells from A2.1+ patients. Consistent with the expression pattern of both transcription factors beyond malignant and nonmalignant plasma cells, PRDI-BF1- and XBP-1-specific CTL activity was not entirely limited to MM targets, but was also associated with lysis of certain other malignancies and, in defined instances, with low-to-intermediate level recognition of a few types of normal cells. Our results also indicate that the A2.1-restricted, PRDI-BF1- and XBP-1-specific human CD8+ T cell repertoire is affected by partial self tolerance and may thus require the transfer of high-affinity TCR to break tolerance. We conclude that transcription factors governing terminal cellular differentiation may provide MM- and tumor-associated CTL epitopes. The Journal of Immunology, 2005, 175: 1301–1309.

The outcome of the majority of patients with multiple myeloma (MM) is unsatisfactory, although a proportion of patients benefit from high-dose therapy followed by autologous stem cell support (1). Allogeneic stem cell transplantation may result in higher complete response rates and, occasionally, in long-term survivors in true molecular remissions (1). This procedure, however, has yet been associated with substantial transplant-related mortality in MM patients (2). The advantages of allogeneic stem cell transplantation are the absence of MM cells in the graft and the existence of a CD8+ T cell-based graft-vs-myeloma effect (3–5). Clinical results, apart from donor lymphocyte infusions (3, 5–7), on a more specific CTL-mediated immunotherapy for MM are solely derived from vaccination trials using the Ig Id (8). The Id represents a private MM-associated Ag and thus cannot provide shared immunotherapy for various MM patients. Nonindividual MM-associated Ag, such as the cancer germline-specific MAGE/BAGE/GAGE/LAGE family gene products or mucin 1 (MUC1), have been identified (9). The expression pattern of MAGE-type genes, however, covers only a proportion (10–50%) of MM cells (9, 10). Although MUC1 has been reported to be expressed in 60–90% of MM samples (9, 11), only low-level cytotoxicity (20–30% specific lysis at E:T ratios of up to 100:1) in response to MM cells has been demonstrated for HLA-A*0201 (A2.1)-restricted CTL specific for the MUC1-derived peptide Ag M1.1 and M1.2 (11, 12). Hence, the identification of novel, MM-associated, MHC class I-presented CTL epitopes remains an important challenge. Moreover, Ag derived from proteins involved in the malignant phenotype are believed to be particularly useful for targeted anti-MM immune interventions, as the escape of neoplastic cells from immune recognition by the selection of Ag-loss variants would be less likely to occur (13, 14).

Naïve B cells initiate a series of temporally and spatially regulated events that lead to the differentiation of both memory B cells and Ab-secreting plasma cells. Two transcription factors, the X box-binding protein 1 (XBP-1) and the mouse B lymphocyte-induced maturation protein 1 (Blimp-1) or its human homologue, the positive regulatory domain I-binding factor 1 (PRDI-BF1), have been recently demonstrated to be essential for terminal plasma-cytic differentiation (15). XBP-1 was found to be required for the plasma cell phenotype, as no plasma cells develop in its absence (16). Blimp-1 has the unique ability to drive plasmacytic differentiation upon enforced expression in a B cell lymphoma cell line (17) or primary splenic B cells (18), and is found in all plasma cell types in vivo (19). More recently, an essential role for the transcription...
factor Blimp-1 in plasma cell differentiation and preplasma memory B cell formation has been defined (20). As with terminally differentiated nonmalignant plasma cells, MM cells have likewise been demonstrated to (over-)express mRNA and protein of XBP-1 and PRDI-BF1 (21–24). Interestingly, however, PRDI-BF1 was found to be involved in additional terminal differentiation processes distinct from the B cell lineage (25), and XBP-1 expression did not appear to be exclusively specific for malignant and nonmalignant plasma cells (26).

In this study, we report on a novel concept for the immunotherapy of MM and other malignancies based on the induction of PRDI-BF1- and XBP-1-specific CTL. By circumventing self tolerance in A2.1 transgenic (Tg) mice (13, 14, 27), we identified several endogenously processed, A2.1-presented PRDI-BF1- and XBP-1-derived CTL epitopes. We show the entire response pattern of Tg mice-derived CTL, including the recognition of primary MM cells, malignant melanoma, breast, and hepatocellular cancer targets. Our results also indicate that the human T cell repertoire is affected by partial PRDI-BF1- and XBP-1-specific self tolerance.

Materials and Methods

Mice

Tg mice (28) were kindly provided by L. A. Sherman (The Scripps Research Institute (TSRI), La Jolla, CA). Homozygous line cA2K, as compared with A2K+, mice, was derived from a different founder animal and had a 3-fold higher transgene expression. C57BL/6 mice were purchased from the breeding colony of the Johannes Gutenberg-University (JGU). Mice were maintained at the animal facility of JGU. Experimental procedures were performed according to German federal and state regulations and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Peptides


Cells

MM cell lines were the A2.1 U266 (American Type Culture Collection (ATCC) TIB 196), DEPU (LB-1696) (kindly provided by N. von Baren, Ludwig Institute for Cancer Research, Brussels, Belgium), L363, OPM-2 (14), and A2.1 NCI-H929 (ATCC CRL-9068). A2.1 EBV-transformed B-lymphoblastoid cell lines (B-LCL) were SY (31), MC-CAR (ATCC CRL-8083), and JY, and IM9 (14). Pre-B acute leukemia cell lines (pre-B-ALL) were the A2.1 EU3 and UoC-B11 (14), and the A2.1 EU1 and UoC-B1 (14). A2.1 tumor lines were the osteosarcoma U2OS (14), the colorectal cancer SW480, the breast cancers BT549 and MCF7 (13), the cervix carcinoma CaSk (ATCC CRL 1550), the hepatocellular carcinoma Heg2 (ATCC HB-8065), and the melanomas Malme 3M (ATCC HTB-64) and NA8-Mel (31), kindly provided by T. Wölfel (JGU). The TAP-deficient cell line T2 and the mouse thymoma EL4 have been described (13). K562 (ATCC CCL-243) were used as NK cell-sensitive target.

Monoocyte-derived mature dendritic cells (DC) were generated under serum-free conditions as described (14, 31). The maturation stage of DC was ascertained by flow cytometry including anti-CD83 staining. Isolated PBMC were poly cloning activated for 3 days in the presence of anti-CD3/CD28 Dynabeads (Dynal) and human rIL-4 at 100 U/ml and cyclosporin A for 3 wk at 5.5 × 10−7 M. Cells were seeded on irradiated (60 Gy) NIH3T3-CD154 transfectants and restimulated every 3–5 days. Growth and activation of CD154-CD40-induced B cells was tested weekly by flow cytometry. Primary MM cells were obtained from bone marrow (BM) samples of patients with MM after informed consent. Briefly, plasma cells were isolated from mononuclear cells with anti-human CD138 microbeads (Miltenyi Biotec) using the AutoMACS separation system (Miltenyi Biotec). Plasma cell purity of >90% was confirmed by flow cytometry.

Tg mouse and DC-stimulated human T lymphocytes were generated as reported (31). For the induction of human T cells stimulated with autologous CD154-CD40-activated B cells, PBMC following adherence were cultured at a 4:1 ratio in the presence of autologous serum, rIL-7 at 50 U/ml (days 1–21), rIL-7 at 50 U/ml plus rIL-2 at 100 U/ml (days 21–28), or rIL-2 at 100 U/ml (days 28) with 30 Gy-irradiated B cells that had been loaded with the indicated peptides at 10−5 M. The derivation of an A2.1-restricted CTL line specific for FluM1 (58–66) (CTL CD8 × A2K+ Flum1) has been described (31). An allorative A2.1-specific CTL line (CTL CD8 Allo A2) was established as reported (13). CTL were used as effector cells in 4–6 h standard 51Cr-release and ELISPOT assays. Cold target inhibition was performed by adding cold T2 cells loaded with either the relevant or an irrelevant control peptide at 10−3 M to hot targets at a cold:hot target ratio of 20:1 before incubation with the CTL. As indicated, target cells were preincubated with the anti-A2 mAbs MA2.1 or PA2.1 (13).

Peptide binding to A2.1

A competition assay (13) was used to assess binding of synthetic PRDI-BF1 and XBP-1 peptides to A2.1. T2 cells were pulsed with 0.01 μg of the A2.1-binding peptide p53 (264–272) and either 3 or 10 μg of PRDI-BF1/ XBP-1 peptides. The A2.1-restricted p53 (264–272)-specific CTL line, CD8 × A2 264, was assayed at various E:T ratios for lytic activity in response to peptide- and nonpeptide-pulsed T2 targets in a 4 h 51Cr-release assay. Percent inhibition of CD8 × A2 264 CTL-mediated lysis of p53 (264–272)–pulsed T2 cells by the PRDI-BF1/XBP-1 peptides was calculated at an E:T ratio of 1:1. The H-2Kb-binding VSV-N (52–59) and the A2.1-binding Flum1 (58–66) peptides served as negative and positive control, respectively.

Inhibiting proteasomal Ag production

OPM-2 cells were washed in HBSS and exposed for 1 min to 0.13 M citric acid/0.061 M NaHPO4 (pH 3.0) (14, 32). Cells were washed in RPMI 1640 and cultured for 5 h with or without lactacytin (Boston Biochem) at 10 and 30 μM. Cells were used as targets in a 5.5 h 51Cr-release assay in the continuous presence or absence of lactacytin (14, 32).

ELISPOT assay

IFN-γ secretion by human and murine effector T cells was determined by ELISPOT assays that take advantage of anti-IFN-γ capture mAb (human IFN-γ: 1D1K; Mabtech/mouse IFN-γ: RMMG-1; Biosource Europe) and detection mAb (human IFN-γ: 7-B6-1; Mabtech/mouse IFN-γ: XMG 12; BD Biosciences) (33). The number of IFN-γ spots was determined by an automated and computer-assisted video-image-analysis (Zeiss-Kontron). Briefly, DC-activated human effector T cells were cocultured under serum-free conditions at 0.3 or 1 × 105 cells/well with 0.5 × 105 target cells. Ag specificity of human T cells was evaluated by adding test or control peptides at a final concentration of 10−3 M. PHA (2 μg/ml) supplemented cocultures served as additional positive controls. Human B cell-stimulated T cells at 6 × 104, 2 × 104, and 1 × 104 were tested in response to 3 × 105 T2 cells loaded with PRDI-BF1 and XBP-1 peptides at various concentrations. BM-derived CD138+ MM cells from A2.1+ MM patients served as target cells in mouse IFN-γ-ELISPOT assays and were cocultured at 1 × 105 cells/well with 3 and 1.5 × 105 Tg mouse CTL. Normal CD 138− cells of the same patient-derived BM sample were adjusted at corresponding target numbers and used as control.

Real-time PCR analysis

Real-time analysis was performed using the ABI 7900HT (Applied Biosystems) with Absolute QPCR SYBR Green Rox Mix (Abgene) according to the manufacturer. Total RNA was isolated with an RNasey Mini kit (Qiagen) from the indicated normal tissues and various tumor samples. Random-primed cDNA synthesis was performed using Superscript II (Invitrogen Life Technologies). For PRDI-BF1 and XBP-1 amplifications, the following primers were designed: PRDI-BF1 sense: 5′-GTACGACCTT GGCCTGCCC-3′; PRDI-BF1 reverse: 5′-CCGCCATCAGCACCAGAAA-3′.
XBP-1 sense: 5′-TTAGAAGAAGAGACCACCATAC-3′; XBP-1 reverse: 5′-ACGTAGTCTGAGTGT-3′. The amount of cDNA was determined by measuring the 18S levels of each sample (34). Relative expression values have been calculated using the ΔΔ threshold cycle (CT) method.

Results

Generating PRDI-BF1- and XBP-1-specific CTL in Tg mice

Based on consensus motifs and computational score models for A2.1-binding peptides (35, 36), we selected a total of 61 and 16 synthetic peptides corresponding to PRDI-BF1 and human XBP-1 protein sequences, respectively. Almost all of the PRDI-BF1 (32 of 44) and XBP-1 (11 of 13) peptides with strong-to-intermediate A2.1-binding capacity were tested for their ability to induce A2.1-restricted and peptide-specific CTL in A2.1 Tg mice to bypass potential self tolerance (data not shown). Immunogenic peptides representing PRDI-BF1 (377–386, 401–410, 402–410, 406–414, 406–415) as well as XBP-1 (18–27) and (19–27) aa were non-homologous to mouse self-PRDI-BF1/-XBP-1 sequences. These peptides had strong A2.1-binding efficiencies, except PRDI-BF1 (406–414) that bound to A2.1 with intermediate activity (Fig. 1).

FIGURE 1. A2.1-binding activity of immunogenic PRDI-BF1 and XBP-1 peptides. A, The A2.1-binding activity, shown as percent inhibition, was determined by the ability of the indicated synthetic peptides to inhibit the A2.1-binding of the p53 (264–272) peptide. This was measured as inhibition of lysis by p53 (264–272)-specific CTL of T2 cells loaded with the corresponding p53 peptide and different concentrations of indicated PRDI-BF1 and XBP-1 peptides (as shown in B). Four experiments are summarized. Representative results are shown for the control competitor peptides, FluM1 (58–66) and VSV-N (52–59). The levels of control lyses in the presence of the antigenic p53 peptide but absence of competitor peptide (T2 + p53) and in the absence of any peptides (T2) are indicated.

Synthetic peptide variants, in which the cysteine side chain was converted into a nonreactive form, suggested that cysteine modification based on oxidation of the free S-H group (37) has an impact on PRDI-BF1-specific CTL recognition. Although we found a 10-fold increase in peptide sensitivity of PRDI-BF1 (401–410) and (402–410)-specific CTL under reducing conditions, no effect was observed for PRDI-BF1 (377–386)-reactive CTL or control CTL recognizing a non-cysteine-containing Ag (data not shown). By loading PRDI-BF1 peptide substitutes in which cysteine was replaced by alanine onto T2 targets, we found that PRDI-BF1 (377–386)- and (402–410)-specific CTL, effector T cells propagated with PRDI-BF1 (401–410), demonstrating a more profound lytic activity in response to the 9-mer PRDI-BF1 (402–410) than to the 10-mer (401–410) (Fig. 2, B).

FIGURE 2. Specificity, A2.1 restriction, and efficiency of Ag recognition by PRDI-BF1 and XBP-1 peptide-reactive CTL. CTL lines established from primary cultures of A, PRDI-BF1 (377–386)-, B, (401–410)-, C, (402–410)-, D, XBP-1 (18–27), and E, (19–27)-induced T cells were tested at various E:T ratios (shown: 10:1) for recognition of T2 targets pulsed with the stimulating peptide (○), the indicated peptide variant (■), or FluM1 (58–66) (▲), and EL4 cells coated with the stimulating Ag (△) or an unrelated A2.1-binding peptide (△) at the indicated concentrations in a 5.5 h 51Cr-release assay.
T2 targets (Fig. 2, D and E). We ascertained the Ag specificity and A2.1 restriction of all CTL lines based on their failure to lyse T2 cells loaded with the A2.1-binding FluM1 (58–66) peptide, and their lack of recognition of A2.1-negative EL4 targets coated with either stimulatory peptide (Fig. 2).

Targeting MM cells by PRDI-BF1- and XBP-1-specific CTL

MM cells have been demonstrated to (over-)express PRDI-BF1 and XBP-1 mRNA and protein (data not shown) (21–24, 38). To assess whether the antigenic PRDI-BF1 and XBP-1 peptides represent naturally processed, A2.1-presented CTL epitopes, we explored MM targets for recognition by PRDI-BF1- and XBP-1-specific CTL. We found that A2.1+ as opposed to A2.1− (NCI-H929) MM cell lines were selectively killed by PRDI-BF1- and XBP-1-reactive CTL (Fig. 3, A–E). Effector T cells with specificity for PRDI-BF1 (402–410) were able to efficiently recognize the A2.1+ MM targets U266, OPM-2, L363, and DEPU (Fig. 3, 3A). Although PRDI-BF1 (401–410)- and (402–410)-reactive CTL were of almost equivalent avidity in response to the 9-mer (402–410) (Fig. 2, B and C), they differed substantially in their functional recognition of A2.1+ MM targets (Fig. 3, B and C). This finding would be consistent with the possibility of a differential sensitivity of these CTL to cysteine modified Ag (Fig. 2C and data not shown) (39).

XBP-1 (19–27)- as compared with (18–27)-specific CTL gave rise to more robust A2.1-restricted lytic responses to the majority of A2.1+ MM targets (Fig. 3, D and E). Although mutual cross-recognition of the 9- and 10-mer peptide by XBP-1 (18–27)- and (19–27)-reactive CTL, respectively, occurred only with anti-XBP-1 (18–27) effector T cells, this ability vanished upon their prolonged in vitro maintenance and was accompanied by impaired recognition of natural Ag presented on MM targets (data not shown). However, their avidity in response to the stimulatory Ag XBP-1 (18–27) itself was not affected. These findings indicate that XBP-1 (19–27) as opposed to (18–27) is the naturally processed CTL epitope.

Lack of killing of A2.1− NCI-H929 targets by PRDI-BF1- and XBP-1-reactive CTL (Fig. 3, A–E) as well as inhibition of the cytolytic response to A2.1− MM cells in the presence of an anti-A2 mAb (Fig. 3, A, C, and E) revealed that MM recognition by PRDI-BF1- and XBP-1-specific CTL was in fact A2.1-restricted. Positive and negative responder controls for MM cell recognition were allo-A2.1-reactive and FluM1 (58–66)-specific T cell lines, respectively (Fig. 3, F and G). We conclude that defined PRDI-BF1- and XBP-1-derived peptides provide naturally processed, MM-associated CTL epitopes.

Peptides presented by class I MHC molecules are most often derived from proteolytic processing of cellular proteins by the 20S proteasomal complex (40). To study whether the epitopes recognized by PRDI-BF1- and XBP-1-specific CTL had been generated by the proteasome, proteasomal Ag production by OPM-2 cells was modified by lactacystin (32). Naturally presented peptides were stripped off cell surface class I MHC molecules of OPM-2 cells by acid treatment to allow only novel peptides generated by proteolytic degradation to be presented for CTL recognition (14, 32). Production of the MM-associated model CTL epitopes PRDI-BF1 (402–410) and XBP-1 (19–27) was inhibited by exposing OPM2 to lactacystin (Fig. 4, A and B), as was lysis by peptide-dependent allo-A2.1-reactive control CTL (Fig. 4D). No effect was
observed with CTL CD8 × A2Kb FluM1 (58–66) used as a negative control (Fig. 4C), indicating that the treatment of OPM-2 targets with the proteasome inhibitor per se did not render them susceptible to CTL-mediated cell death. These findings demonstrate that the natural PRDI-BF1 and XBP-1 peptides recognized by CTL were endogenously processed by the proteasome complex.

To extend the analysis of PRDI-BF1- and XBP-1-specific CTL to primary MM cells, BM samples from A2.1+ MM patients were separated into the CD138− MM subset and normal CD138+ BM mononuclear cells. PRDI-BF1- and XBP-1-specific CTL produced IFN-γ in response to freshly isolated MM as opposed to normal BM stimulators (Fig. 5A). Allo-A2.1- and FluM1 (58–66)-reactive CTL were used as positive and negative responder control, respectively. MM-specific cytotoxic activity was only observed with PRDI-BF1 (402–410) and (401–410)-specific T cells, albeit less efficiently (Fig. 5B). Comparable results were obtained with BM samples from four of six MM patients. Obviously, primary MM cells freshly isolated from A2.1+ patients are targeted by PRDI-BF1- and XBP-1-reactive effector T cells. Lack of recognition of CD138− BM targets also precludes the possibility that PRDI-BF1- and XBP-1-reactive T lymphocytes cross-recognized other non-MM cell-derived peptides or responded by peptide-dependent allorecognition.

PRDI-BF1- and XBP-1-specific CTL activity beyond MM targets

PRDI-BF1-/XBP-1-targeted immunotherapy would ideally be associated with selective killing of malignant targets and, perhaps, nonmalignant plasma cells while preserving other nontransformed tissues. Recent observations, however, have indicated a broader expression pattern of PRDI-BF1 than has so far been anticipated, arguing for its involvement in additional terminal differentiation processes distinct from the B cell lineage (25). XBP-1, too, has been found expressed in other tissues, including the liver (16, 41). These findings led us to study PRDI-BF1- and XBP-1-specific CTL in response to other non-MM-related B cell malignancies and B-LCL, various solid tumor cells, and nontransformed targets (Fig. 6). FluM1 (58–66)- and allo-A2.1-reactive effector T cells were used as controls (data not shown).

PRDI-BF1-reactive CTL responded to the majority of EBV-transformed B-LCL, the PRDI-BF1+ osteosarcoma line U2OS (42), and the melanoma targets NA8-Mel and Malme 3M, whereas pre B-ALL were mostly ignored (Fig. 6, A–C). These results are in accordance with expression analyses of the mouse homologue of PRDI-BF1, Blimp-1 (17). The differential capacity of PRDI-BF1-specific CTL to respond to transformed A2.1+/PRDI-BF1+ cells, as shown in Figs. 3, A–C, and 6, A–C, was also observed with nontransformed targets. Whereas all normal cells were ignored by PRDI-BF1 (377–386)-reactive effector T cells (Fig. 6A), low level recognition of mature DC (25% lysis) by CTL PRDI-BF1 (402–410) in one of three experiments and of PBMC (20% lysis) by CTL PRDI-BF1 (402–410) occurred (Fig. 6, B and C). PRDI-BF1 (402–410)-specific CTL gave also rise to ~45% specific lysis of purified B cells (Fig. 6C). Interestingly, formation of (preplasma) memory B cells has been demonstrated more recently to require the expression of Blimp-1 (20). Taken together, the overall pattern of cytolytic responses revealed for the different PRDI-BF1-specific CTL (Figs. 3, A–C, and 6, A–C) seemed to be cogoverned by their individual avidity (Fig. 2, A–C).

Effector T cells with specificity for XBP-1 (18–27) and (19–27) did not distinguish between different B cell malignancies and efficiently killed all A2.1+ EBV-transformed B-LCL and pre B-ALL lines while ignoring the A2.1+ pre B-ALL targets, EU1 and UoC-B1 (Fig. 6D and data not shown). XBP-1-reactive CTL also recognized NA8-Mel and Malme 3M melanoma cells, whereas additional cytolytic activity in response to breast cancer (BT549) and hepatocellular carcinoma (HepG2) targets was only revealed for XBP-1 (19–27)-specific CTL (Fig. 6D and data not shown). Lysis of HepG2 cells by XBP-1 (19–27)-stimulated CTL is consistent with XBP-1 expression in hepatocellular carcinomas (43). Its level of expression in adult human liver (43), however, was not sufficient to allow XBP-1-specific killing of normal A2.1+ hepatocytes isolated from two different sources (Fig. 6D and data not shown). The differential avidity of XBP-1-reactive CTL in recognizing the (19–27) epitope became more apparent in their responses to other nontransformed cells. Although normal targets were ignored by XBP-1 (18–27)-reactive CTL, XBP-1 (19–27)-specific effector T cells demonstrated intermediate level killing of A2.1+ mature DC (40% lysis) (Fig. 6D and data not shown). Mature DC are believed to be protected from CTL-mediated apoptosis by expression of the granzyme inhibitor, serine protease inhibitor-6 (44). The observed anti-DC T cell responses could be due to the readout via the 51Cr-release as opposed to other assays, such as JAM, that are based on DNA fragmentation (44, 45).

Inhibition of the cytolytic response to the A2.1+ melanoma cell line Malme 3M and purified B cells in the presence of an anti-A2
A mAb (Fig. 6, C and D) revealed that recognition of non-MM tumor targets and nontransformed B cells by PRDI-BF1- and XBP-1-specific CTL was indeed A2.1-restricted. Cold target inhibition of MM cell killing by PRDI-BF1- and XBP-1-reactive CTL (Fig. 6) as well as B cell and mature DC recognition by XBP-1-reactive CTL (Fig. 6D) again demonstrated the Ag specificity of these effector T cells and precludes the possibility that PRDI-BF1- and XBP-1-reactive CTL cross-recognized other non-PRDI-BF1- or non-XBP-1-derived peptides.

Based on these results and the expression patterns of PRDI-BF1 and XBP-1 (16, 17, 20, 25, 26, 38, 41), we conclude that PRDI-BF1- and XBP-1-reactive CTL activity is not entirely limited to MM targets, but is also associated with lysis of other malignancies and, in the case of PRDI-BF1 (401–410)-, (402–410)-, and XBP-1 (19–27)-, but not PRDI-BF1 (377–386)-specific CTL, with low-to-intermediate level recognition of a few types of nontransformed cells.

Consistent with these observations, we found expression of PRDI-BF1 and XBP-1 transcripts in a number of normal tissues (Fig. 7). However, expression levels were 4-fold lower as compared with a MM standard (NCI-H929) (Fig. 7). XBP-1 CTL-mediated lysis of BT549 and HepG2 as opposed to normal hepatocytes (Fig. 6D) seemed to correlate with elevated XBP-1 transcripts in breast and hepatocellular cancer relative to normal breast and liver (Fig. 7). The PRDI-BF1 and XBP-1 RNA expression in normal bone marrow (Fig. 7), however, was not associated with susceptibility to Ag-specific CTL recognition (Fig. 5).

Partial self tolerance to PRDI-BF1 and XBP-1 in the human CD8+ T cell repertoire

Supported by these findings, we wanted to confirm our prejudice that the human CD8+ T cell repertoire is affected by PRDI-BF1- and XBP-1-specific self tolerance. To this end, naive CD8+ T
lymphocytes from at least two different A2.1+ healthy donors underwent in vitro stimulation with mature autologous DC that had been pulsed with either of the three relevant PRDI-BF1 Ag, XBP-1 (19–27), or the hTyr (369–377) control peptide. By using PRDI-BF1 (377–386) and (402–410) for CTL induction, the generated CD8+ T cell cultures were neither cytolytic nor did they secrete IFN-γ in response to peptide-coated T2 targets (data not shown). In contrast, CD8+ T cells able to specifically secrete IFN-γ upon challenge with peptide-loaded T2 targets (Fig. 8A). The failure to promote CTL in nonresponding cultures was due to the use of PRDI-BF1 and XBP-1 peptides as hTyr (369–377)-specific and malignant melanoma-reactive cytolytic effector T cells were generated in all donors (Fig. 8A and data not shown) (31). As the number of PRDI-BF1 (401–410)- and XBP-1-reactive, DC-induced human CD8+ effector T cells was limited and their avidity appeared to be low to intermediate, we tested their capability of recognizing MM targets in the IFN-γ ELISPOT assay. Likewise, only donor 1-derived CTL

![Figure 8](http://www.jimmunol.org/)

**FIGURE 8.** Specificity, A2.1-restriction, and anti-MM activity by human CD8+ T cells induced with PRDI-BF1 (401–410) and XBP-1 (19–27). Human CD8+ T lymphocytes isolated from at least two A2.1+ healthy donors and stimulated in parallel for five cycles with (left panel) PRDI-BF1 (401–410)-, (middle panel) XBP-1 (19–27)-, and (right panel) control hTyr (369–377)-pulsed autologous mature DC were tested for IFN-γ secretion at the indicated E:T ratios in response to A, T2 cells coated with the indicated peptides at 10^{-5} M. B, PRDI-BF1 (401–410)- and XBP-1 (19–27)-induced CD8+ T cells from donor 1 and the due control stimulated with hTyr (369–377) were assayed for recognition of the A2.1+ MM cell lines L363 and U266, the A2.1+ MM target NCI-H929, and the NK-sensitive K562 line.

HIV-RT peptide-coated targets (Fig. 8A). Specific killing of Ag-pulsed T2 cells was only observed for PRDI-BF1 (401–410)- and XBP-1 (19–27)-reactive CTL obtained from donor 1 (100 and 30% specific lysis at an E:T of 20:1, respectively), and required higher peptide concentrations (10^{-5} M) as compared with TG mouse CTL (Fig. 2, B and E). The failure to promote CTL in nonresponding cultures was due to the use of PRDI-BF1 and XBP-1 peptides as hTyr (369–377)-specific and malignant melanoma-reactive cytolytic effector T cells were generated in all donors (Fig. 8A and data not shown) (31). As the number of PRDI-BF1 (401–410)- and XBP-1-reactive, DC-induced human CD8+ effector T cells was limited and their avidity appeared to be low to intermediate, we tested their capability of recognizing MM targets in the IFN-γ ELISPOT assay. Likewise, only donor 1-derived CTL

![Figure 9](http://www.jimmunol.org/)

**FIGURE 9.** Human PRDI-BF1- and XBP-1-specific T cell responses. Human T cells from 10 different A2.1+ healthy donors were stimulated with CD154-CD40-activated autologous B cells that had been loaded with either of the PRDI-BF1 377 and 401 peptides or the XBP-1 19 Ag. The IFN-γ release of the 5 of 10 donor T cells that responded specifically to either the entire (donor 1) or a part of the MM peptide panel (donors 2–5) is shown. Results represent responses to T2 stimulators at the lowest Ag concentration tested (10^{-7} M) and to A2.1+ L363 and U266 MM targets. HIV-RT peptide-pulsed T2 and the A2.1+ MM target NCI-H929 served as negative controls. The number of IFN-γ spots is calculated per 1 × 10^5 responder T cells.
were capable of specifically secreting IFN-γ in response to the A2.1* MM cell lines L363 and U266 (Fig. 8B). MM cell recognition was A2.1-restricted and not affected by NK cell activity as no response to A2.1* NCI-H929 and K562 targets occurred (Fig. 8B). Corresponding effector T cells stimulated with hTyr (369–377) served as negative control (Fig. 8B).

To confirm these findings, human T cells from 10 different A2.1* healthy donors were stimulated with CD154-CD40-activated autologous B cells that had been loaded with either of the PRDI-BF1 377 and 401 peptides or the XBP-1 19 Ag. Five of 10 donor T cells responded by IFN-γ secretion in the presence of PRDI-BF1 377 and 401 peptides or the XBP-1 19 Ag. Five of 10 activated autologous B cells that had been loaded with either of the PRDI-BF1- and XBP-1-specific human CD8+ T cells responded by IFN-γ secretion. Careful evaluation of the effect of Blimp-1- and mouse IRF4 (57). Therefore, we propose that IRF4 is likely to serve as a new class of proteins, defined transcription factors, to tackle MM in suitable preclinical mouse models is necessary to address this concern. However, normal BM cells were not recognized by the due CTL and minority of cells expressing low level and the vast majority of cells expressing no Blimp-1 at all (48). Yet, the broader pattern of PRDI-BF1- and XBP-1-specific target cell recognition could nevertheless be accompanied by the risk of autoimmune T cell responses. Careful evaluation of the effect of Blimp-1- and mouse XBP-1-specific T cells in suitable preclinical mouse models is necessary to address this concern.

Our results suggest that the A2.1-restricted, PRDI-BF1- and XBP-1-specific human CD8+ T cell repertoire, in contrast to A2.1 Tg mice, is affected by partial self tolerance. It was by far more difficult to obtain a human T cell response to these peptides, consistent with an effect on the repertoire due to partial self tolerance. Provided that autoimmunity is controlled, limited to defined tissues, or absent (as indicated for PRDI-BF1 (377–386)), the findings reported here offer the appealing therapeutic possibility of turning human T lymphocytes into efficient MM- and tumor-reactive CTL by gene transfer of PRDI-BF1- and XBP-1-specific TCR (49), as has been recently demonstrated in our laboratory with A2.1-restricted, MDM2 (81–88)- and p53 (264–272)-specific TCR obtained by circumventing self tolerance in Tg mice (14, 50). Allo-MHC-restricted TCR (51), in vitro mutated and selected TCR of high affinity (52, 53), and TCR-like molecules (54) are due therapeutic instruments of human origin (55).

Finally, the presented experiments provide the basis to consider a new class of proteins, defined transcription factors, to tackle MM and other malignancies. Apart from PRDI-BF1 and XBP-1, another transcription factor, IFN regulatory factor 4 (IRF4), also known as MM oncogene 1 (MUM1), was shown to be inherently involved in plasmacytic differentiation (56). Recent studies reported on the recurrent genetic aberration in MM that juxtaposes the IgH locus to the IRF4 gene, resulting in the overexpression of IRF4 (57). Therefore, we propose that IRF4 is likely to serve as another candidate MM-associated Ag. Gene expression profiling in plasma cells revealed that these cells display a highly specialized genetic program including the expression of unique sets of known and novel transcription factors (24, 58) that may also provide novel target molecules for CTL-based immunotherapy of MM.

Disclosures
The authors have no financial conflict of interest.

References


