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Immunology

T-cell immunity to the joining region of p210$^{BCR-ABL}$ protein

(chronic myelogenous leukemia/chromosome translocation)

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ABSTRACT **The hallmark of chronic myelogenous leukemia is the translocation of the human c-*abl* protooncogene (*ABL*) from chromosome 9 to the specific breakpoint cluster region (*bcr*) of the *BCR* gene on chromosome 22. The t(9;22)(q34;q11) translocation results in the formation of a *BCR–ABL* fusion gene that encodes a 210-kDa chimeric protein with abnormal tyrosine kinase activity. The *ABL* and *BCR* genes are expressed by normal cells and thus the encoded proteins are presumably nonimmunogenic. However, the joining-region segment of the p210$^{BCR-ABL}$ chimeric protein is composed of unique sequences of ABL amino acids joined to BCR amino acids that are expressed only by malignant cells. The current study demonstrates that the joining region of BCR–ABL protein is immunogenic to murine T cells. Immunization of mice with synthetic peptides corresponding to the joining region elicited peptide-specific, CD4$^+$, class II major histocompatibility complex-restricted T cells. The BCR–ABL peptide-specific T cells recognized only the combined sequence of BCR–ABL amino acids and not BCR or ABL amino acid sequences alone. Importantly, the BCR–ABL peptide-specific T cells could recognize and proliferate in response to p210$^{BCR-ABL}$ protein. The response of peptide-specific T cells to protein demonstrated that p210$^{BCR-ABL}$ can be processed by antigen-presenting cells so that the joining segment is bound to class II major histocompatibility complex molecules in a configuration similar to that of the immunizing peptide and in a concentration high enough to stimulate the antigen-specific T-cell receptor. Thus, BCR–ABL protein represents a potential tumor-specific antigen related to the transforming event and shared by many individuals with chronic myelogenous leukemia.**

The t(9;22)(q34;q11) translocation is exceedingly consistent and is present in >95% of patients with chronic myelogenous leukemia (CML) (reviewed in ref. 1). The translocation of the c-*abl* protooncogene (*ABL*) on chromosome 9 to the breakpoint cluster region (*bcr*) on chromosome 22 forms a fusion gene termed *BCR–ABL*, which encodes a 210-kDa chimeric protein with abnormal tyrosine kinase activity (2–7). p210$^{BCR-ABL}$ can stimulate the growth of hematopoietic progenitor cells and is essential for the pathogenesis of CML. In CML, the *BCR* breakpoint is generally between exons 2 and 3 or exons 3 and 4. Depending on the location of the breakpoint within the *BCR* gene, the mRNA either includes or excludes *BCR* exon 3. Regardless of the location of the breakpoint, the *BCR–ABL* reading frames are fused in frame and the translocated mRNA encodes a functional 210-kDa chimeric protein consisting of 1004 *ABL*-encoded amino acids plus either 902 or 927 *BCR*-encoded amino acids; both of these chimeric proteins are enzymatically active as protein kinases (8, 9). In acute lymphoblastic leukemia (ALL), *ABL* is translocated to chromosome 22 but to a different region of the *BCR* gene, denoted *BCR1* (10), which results in the expression of a p185–190$^{BCR-ABL}$ chimeric protein kinase (11–13). p185–190$^{BCR-ABL}$ is expressed in approximately 10% of children and 25% of adults with ALL.

In animal models, disseminated leukemia can be eradicated by the use of T cells immune to retrovirus-encoded leukemia-associated antigens (14, 15). A major obstacle preventing successful application of similar therapy to humans is a lack of well-characterized antigens that are both uniquely expressed on malignant cells and recognizable by T cells. Theoretically, any protein expressed by an altered cancer-related gene, such as a protooncogene, is a potential T-cell target. This concept is just beginning to be evaluated (16). In general, T cells do not recognize intact proteins but, rather, recognize short peptide fragments, 8–12 amino acids in length, that have been derived from intact protein processed intracellularly and presented in the cleft of major histocompatibility complex (MHC)-encoded molecules. Thus, the appropriateness of any particular protooncogene product to serve as a T-cell target will depend upon whether the altered segment of protein has the proper molecular configuration or "motif" to bind in the cleft of class I or class II MHC molecules; whether the resultant peptide/MHC molecule complex is present at the cell surface of the target cell or antigen-presenting cell (APC) in a concentration high enough to stimulate the specific T-cell receptor (17, 18); and, finally, whether the peptide/MHC molecule complex is within the T-cell receptor repertoire of the particular nominated individuals (17–21). The current study indicates that each of these stringent conditions can be met in mice. Thus, p210$^{BCR-ABL}$ protein can be immunogenic to T cells by virtue of the few novel BCR–ABL amino acids at the joining region. Whether specific T-cell recognition of BCR–ABL protein can occur in humans, and whether BCR–ABL protein is an appropriate target for T-cell therapy, needs to be evaluated.

MATERIALS AND METHODS

Mice. Female BALB/c and C57BL/6 (B6) mice, 6–8 weeks old, were obtained from The Jackson Laboratory.

Tumor Cell Lines, Peptides, and Proteins. K562 is a Philadelphia chromosome (Ph^1)-positive human CML cell line (3). 8B1 and 106-5 are BALB/c murine leukemic cell lines derived by retroviral transformation of BALB/c bone marrow cells with p210 or p185 *BCR–ABL* fusion genes, respectively (22, 23), and kindly provided by O. N. Witte. K-BALB is a Kirsten murine sarcoma virus-transformed BALB 3T3 fibroblast tumor cell line. Synthetic peptides (Table 1) were synthesized by Mary Kay Dojeski at the Fred Hutchinson Cancer Research Center, using a model 430A automated solid-phase peptide synthesizer with FastMoc chemistry (Applied Biosystems). To prepare BCR–ABL protein extracts, K562, 8B1, 106-5, and K-BALB cells were harvested and resuspended in 25 mM potassium phosphate (pH 7.0)

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Abbreviations: ALL, acute lymphocytic leukemia; APC, antigen-presenting cell; CML, chronic myelogenous leukemia; MHC, major histocompatibility complex.

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Table 1. BCR–ABL peptide amino acid sequences

Peptide	No. of residues	Sequence
		↓
BCR3–ABL2	22	IVHSATGFKQSSKALQRPVASD
BCR3–ABL2	18	HSATGFKQSSKALQRPVA
BCR3–ABL2	16	GFKQSSKALQRPVASD
BCR3–ABL2	14	GFKQSSKALQRPVA
BCR3–ABL2	12	GFKQSSKALQRP
BCR3	12	IVHSATGFKQSS
BCR2–ABL2	12	LTINKEEALQRP
BCRI–ABL2	14	AFHGDAQALQRPVA

Amino acids are represented by single-letter code; BCR3–ABL2 denotes sequence from $p210^{BCR-ABL}$ encoded by a fusion gene in which *BCR* exon 3 is fused to *ABL* exon 2; BCR2–ABL2 denotes sequence from $p210^{BCR-ABL}$ encoded by a fusion gene in which *BCR* exon 2 is fused to *ABL* exon 2; BCRI–ABL2 denotes sequence from $p185^{BCR-ABL}$ encoded by a fusion gene in which the *BCRI* region of the *BCR* gene is fused to *ABL* exon 2 (10). Arrow indicates joining point.

with 2 mM EDTA, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 50 μg of leupeptin per ml, 100 units of aprotinin per ml, and 20 mM benzamidine (24). The cells were homogenized by use of ultrasonic cell disrupter (model W-380, Heat Systems/Ultrasonics), the homogenate was centrifuged for 10 min at 3000 × *g*, and the supernatant was centrifuged at 100,000 × *g* for 90 min. The high molecular weight (M_r > 100,000) supernatant proteins in the cell extracts were isolated and concentrated by using a PM100 DiAFLO ultrafilter (Amicon). The partially purified BCR–ABL proteins were immunoprecipitated from the lysate with monoclonal antibody to BCR (Oncogene Science, Manhasset, NY), separated by SDS/PAGE, and identified by Western immunoblotting with antibody to ABL (Oncogene Science). The BCR–ABL proteins were aliquoted and stored at −70°C.

Immunization Protocols and Proliferation Assays. Mice were inoculated twice at 2-week intervals by subcutaneous injection with complete Freund's adjuvant (Sigma), alone or emulsified with BCR3–ABL2 peptide (100 μg). Ten days after the final immunization, single-cell suspensions of draining lymph nodes and spleens were prepared in medium consisting of a 1:1 mixture of RPMI 1640 medium (GIBCO) and EHAA medium (Biofluids, Rockville, MD) with 25 μM 2-mercaptoethanol, 200 units of penicillin per ml, 200 units of streptomycin per ml, 10 mM L-glutamine, and 10% fetal bovine serum. For proliferation assays, lymphocytes were cultured in 96-well plates at 2 × 10^5 cells per well with 4 × 10^5 irradiated (3000 rads; 1 rad = 0.01 Gy) syngeneic spleen cells and the designated peptide (total volume of 200 μl per well). Plates were incubated in a humidified atmosphere under 5% CO_2 tension at 37°C for 72 hr, and then incubated for 18 hr with 1 mCi (37 MBq) of [^{3}H]thymidine per well. For assay of proliferative responses of cloned T cells, each well contained 2 × 10^4 T cells with 5 × 10^5 irradiated (3000 rads) syngeneic spleen cells and the designated peptide.

Generation and Characterization of $p210^{BCR-ABL}$-Specific T-Cell Clones. Lymphocytes from the draining lymph nodes of mice immunized with BCR3–ABL2 peptide were cultured in 24-well culture plates at 4 × 10^6 cells per well with 2 × 10^6 irradiated syngeneic spleen cells and BCR3–ABL2 peptide at 25 μg/ml (total volume, 2 ml). The plates were cultured in a humidified atmosphere under 5% CO_2 tension at 37°C for 5 days and then split 1:2. After 10 days, lymphocytes were restimulated at 1 × 10^6 lymphocytes per well in 24-well culture plates with 5 × 10^6 irradiated syngeneic spleen cells and peptide at 25 μg/ml. Two days after the second *in vitro* stimulation, T cells were plated at 0.5 cell per well in 96-well flat-bottom plates with 1 × 10^6 irradiated syngeneic spleen cells and recombinant interleukin 2 (rIL-2) (at 50 units/ml). Derived T-cell clones were maintained by periodic stimulation with peptide (1 μg/ml) plus irradiated syngeneic spleen cells followed by expansion with rIL-2 (10 units/ml) every 2 weeks. T-cell clones were stained with fluorescein-conjugated anti-Thy-1.2 (1%), fluorescein-conjugated anti-Lyt-2 (2%), or phycoerythrin-conjugated anti-L3T4 (3%) monoclonal antibodies (Becton Dickinson) at 4°C for 45 min.

RESULTS

Synthetic Peptides Corresponding to the Joining Region of $p210^{BCR-ABL}$ Protein Can Elicit T-Cell Responses. BALB/c mice were immunized with a synthetic 12-amino acid peptide identical to the joining region of the p210 protein in K562 CML cells, termed BCR3–ABL2 peptide (Table 1). Immunity was validated by the demonstration that host lymph node and splenic lymphocytes from immunized mice proliferated *in vitro* in response to the immunizing peptide but not control peptides (Fig. 1). Similar data were generated in the B6 mouse strain. The immunizing peptide, GFKQSSKALQRP, contained six amino acids from BCR (GFKQSS), one fusion amino acid (K), and five amino acids from ABL (ALQRP) (Table 1). To examine for fine specificity, the T-cell lines were cloned by limiting dilution and maintained by episodic restimulation with the BCR3–ABL2 peptide. All clones were Thy-1.2$^+$ CD4$^+$ CD8$^-$ by analysis with fluoresceinated antibodies. Depending upon which BCR domains are included, the BCR–ABL chimeric protein takes two major forms in

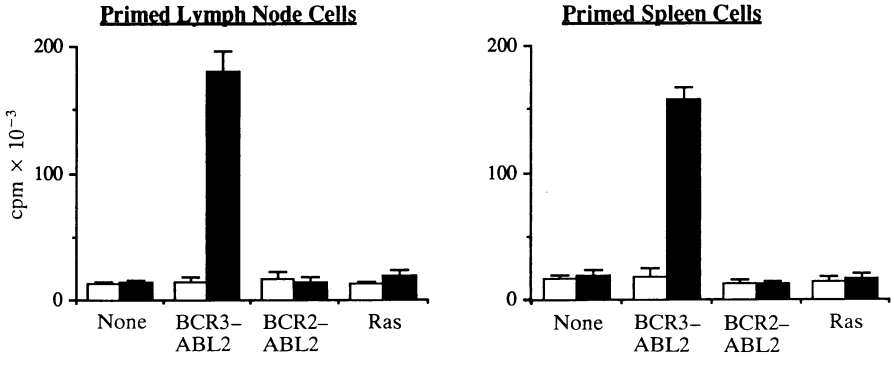

FIG. 1. Lymphocytes from spleens and lymph nodes of BALB/c mice primed *in vivo* with BCR3–ABL2 12-amino acid peptide corresponding to the joining region of $p210^{BCR-ABL}$ chimeric protein proliferate specifically in response to the sensitizing peptide *in vitro*. Ten days after immunization, draining lymph node cells and spleen cells from mice that had been inoculated with adjuvant alone (open bars) or adjuvant plus BCR3–ABL2 12-mer peptide (filled bars) were cultured with medium alone or with BCR3–ABL2 12-mer peptide, or BCR2–ABL2 12-mer peptide, or Ras-c12 12-mer peptide at 25 μg/ml and were assayed after 96 hr for proliferation by [^{3}H]thymidine incorporation. Bars represent the means + SD of triplicate determinations of the cpm.

Table 2. Specificity of CD4+ T-cell clones to a 12-amino acid peptide corresponding to the joining region of the $p210^{BCR-ABL}$ chimeric protein

T-cell clone	[3H]Thymidine incorporation, cpm					
	Medium	BCR3–ABL2	BCR2–ABL2	BCRI–ABL2	BCR3	Ras-c12
2E10	243	242,633	287	137	667	258
1D2	267	274,701	274	315	294	357
3B7	230	64,935	349	277	462	497
2E3	535	293,219	402	461	193	372
3E11	269	263,425	299	398	305	579
3G2	618	30,846	478	583	461	504
3G3	394	61,521	217	351	190	273
2B12	424	109,319	341	385	461	307
2D4	532	262,799	143	147	133	259

Resting CD4+ T-cell clones of BALB/c origin were cultured with irradiated syngeneic spleen cells as APCs plus the indicated peptides at 5 μg/ml. In Tables 2–5, data represent the mean of triplicate determinations of [3H]thymidine incorporated for the final 18 hr of a 96-hr culture. SDs were <10% of the mean cpm.

CML (BCR2–ABL2 and BCR3–ABL2) and one major form in ALL (BCRI–ABL2) (9–13). In each case, the ABL sequence of amino acids is identical but is joined with distinct BCR amino acid sequences. Data from nine representative clones (Table 2) demonstrated that the immune T cells responded specifically to BCR3–ABL2 but not to the alternative BCR–ABL joining regions of BCR2–ABL2 or BCRI–ABL2. The immune T cells likewise failed to respond to peptides identical to the isolated amino acid sequence of BCR3 alone. Thus, the immunogenic determinant was specifically associated with the 12 amino acid residues of the joining region of the $p210^{BCR3-ABL2}$ protein.

The concentration of BCR3–ABL2 peptide necessary to induce proliferation varied. In general, optimal stimulation occurred in the range of 1–5 μg/ml, but some clones responded vigorously to as low as 0.04 μg/ml, the lowest concentration tested (Table 3). Antigen-specific responses of CD4+ T cells require not only binding to class II MHC molecules on APCs but also display of the antigenic epitope in the proper orientation in the MHC cleft. The addition or alteration of flanking amino acids can alter binding, and T cells derived by immunizing with a particular peptide might respond only to that particular peptide. Thus, two BCR3–ABL2-specific T-cell clones derived by immunization with a 12-mer BCR3–ABL2 peptide were tested for response to 14-, 16-, 18-, and 22-mer peptides synthesized to be identical to the naturally occurring amino acid sequences of the p210 joining region. The 12-mer BCR3–ABL2 peptide-specific T cells responded to each of the longer peptides (Table 4), implying that a broad spectrum of joining-region peptides could be appropriately presented by class II MHC molecules.

Table 3. Proliferative response of CD4+ T-cell clones to various concentrations of a 12-amino acid peptide corresponding to the joining region of the $p210^{BCR-ABL}$ chimeric protein

T-cell clone	[3H]Thymidine incorporation, cpm					
	Medium	25	5	1	0.2	0.04
2E10	471	131,643	312,783	333,056	235,103	26,195
1D2	496	66,329	259,018	183,229	91,251	16,423
3B7	421	53,441	149,640	111,212	56,967	60,957
2E3	399	358,253	407,729	273,273	93,921	1,475
3E11	556	40,755	130,633	189,843	135,985	91,240
3G2	561	132,024	168,387	150,799	49,602	9,440
3G3	455	101,280	143,670	93,219	18,403	11,510
2B12	364	132,620	229,010	213,089	71,651	4,132
2D4	387	42,623	182,266	256,606	150,711	12,599

Resting T-cell clones were cultured with irradiated syngeneic spleen cells as APCs plus BCR3–ABL2 12-mer peptide at the indicated concentration [0 (medium) to 25 μg/ml].

Table 4. Proliferative response of CD4+ T-cell clones to BCR3–ABL2 peptides of various lengths

T-cell clone	[3H]Thymidine incorporation, cpm					
	Medium	12-mer	14-mer	16-mer	18-mer	22-mer
2E10	589	96,525	170,085	139,083	224,674	29,509
1D2	159	120,049	238,297	108,309	150,309	21,208

Resting T-cell clones were cultured with irradiated syngeneic spleen cells as APCs plus BCR3–ABL2 peptides (5 μg/ml) (see Table 1 for structures).

BCR3–ABL2 Peptide-Specific T Cells Can Recognize $p210^{BCR-ABL}$ Protein. Peptide-specific T cells often do not respond to proteins containing the same peptide (16). Recognition of a particular peptide amino acid sequence in a larger protein requires that the protein be processed in APCs in a fashion so that the designated peptide segment is bound to self MHC molecules in the appropriate configuration—a circumstance that need not necessarily occur. Therefore, it was essential to determine whether peptide-specific T cells could respond to p210 protein. Partially purified $p210^{BCR-ABL}$ from K562 and 8B1 tumor cells, $p185^{BCR-ABL}$ protein from 106-5 tumor cells, and irrelevant proteins from K-BALB tumor cells were prepared. Several BCR3–ABL2 peptide-specific CD4+ T-cell clones were tested in a proliferation assay with irradiated syngeneic APCs plus either BCR3–ABL2 peptide or partially purified BCR–ABL proteins. BCR3–ABL2 peptide-specific T cells responded to $p210^{BCR-ABL}$ protein, but not to $p185^{BCR-ABL}$ protein or irrelevant K-BALB proteins (Table 5). The proliferative response to $p210^{BCR-ABL}$ was less than the response to peptide, presumably due either to fewer joining-region segments being present in the protein preparation on a per weight basis or to competition for binding to MHC molecules by alternative segments of the protein.

DISCUSSION

The results provide evidence that the generation of T-cell responses to BCR–ABL protein is possible. Immunity was directed specifically against the 12 amino acid residues of the joining region, validating that the $p210^{BCR-ABL}$ protein consisting of 1931 amino acid residues can be processed by APCs so that the joining-region segment is bound in the cleft of class II MHC molecules in a concentration high enough to stimulate T cells, and in a configuration similar to the configuration engendered when mice were immunized with a 12-mer joining-segment peptide. The immunizing peptide was composed of six BCR, one fusion, and five ABL amino acids. The c-*abl* → *bcr* translocation is not known to occur in mice. However, expression of the human *BCR–ABL* gene in mice leads to a malignant syndrome similar to CML (25, 26). The c-*abl*-

Table 5. Proliferative response of BCR3–ABL2 peptide-specific T-cell clone to $p210^{BCR-ABL}$ chimeric proteins

Peptide/protein	[3H]Thymidine incorporation, cpm			
	Medium	1	0.2	0.04
BCR3–ABL2 14-mer peptide	214	92,970	54,896	6,303
$p210^{BCR-ABL}$ from K562	207	6,679	2,270	338
$p210^{BCR-ABL}$ from 8B1	521	2,498	1,483	236
$p185^{BCR-ABL}$ from 106-5	231	157	198	312
Proteins from K-BALB	164	197	276	284

BCR3–ABL2 peptide-specific CD4+ T-cell clone 2E10 (Table 2) was cultured with irradiated syngeneic spleen cells as APCs plus the indicated concentration (μg/ml) of BCR3–ABL2 14-mer peptide, or of partially purified $p210^{BCR-ABL}$ from K562 and 8B1 cells or $p185^{BCR-ABL}$ from 106-5 cells, or of proteins from irrelevant K-BALB tumor cells.

encoded amino acids of the joining region are identical in mice and humans. It is unknown whether the *bcr*-encoded amino acids are expressed normally in mice. Thus, the *bcr* segment might be xenogeneic. However, the immune response generated was not to BCR residues alone. The novel antigenic determinant created by the chimeric protein required both BCR and ABL residues. In humans the BCR–ABL protein is expressed only by leukemic cells. Thus, the joining-region segment is unique to humans as well as mice and similar responses in humans are possible.

Studies by others have shown that analogous BCR–ABL joining-region peptides could elicit specific antibody responses to BCR–ABL proteins (27, 28). Elicitation of an antibody response often requires a concurrent $CD4^+$ helper T-cell response. The current study provides direct evidence that a T-cell response to BCR–ABL joining-region peptides is possible. The importance of the observation that peptide-specific T cells can also respond to BCR–ABL protein is the speculation that BCR–ABL proteins may be appropriate targets for therapeutic T-cell attack. BCR–ABL proteins are appealing candidate targets for immunotherapy for several reasons. (*i*) BCR–ABL protein is an antigen shared by many individuals, being detected in >90% of patients with CML and 13% of patients with ALL (1). (*ii*) BCR–ABL protein is intimately associated with malignant transformation as well as maintenance of the malignant phenotype (27–30). CML is by all evidence a monoclonal expansion of a single aberrant pleuripotent stem cell (31). Although controversial (31, 32), it is likely that $p210^{BCR-ABL}$ protein is present in the earliest progenitor malignant cells. Only rarely does Ph^1-positive CML become Ph^1-negative, and only rarely does Ph^1-positive CML lose detectable *BCR–ABL* transcripts during disease progression (33). Thus, antigen-negative variants are not a perceived problem. (*iii*) T cells in CML are almost always Ph^1-negative, at least in chronic phase, and thus, any autochthonous T-cell lines to be used for therapy would not be derived from the leukemic clone (34). (*iv*) Antigenicity of BCR–ABL protein is presumably restricted to the joining region, which is expressed only by malignant cells or "pre-malignant" cells undergoing transformation. Therefore, therapy would be selective, minimizing the potential for autoimmune toxicity. (*v*) Normal bone marrow precursors commonly coexist with the malignant clone in the bone marrow of CML patients, so that selective destruction of the malignant clone could leave normal hematopoiesis intact.

Whether BCR–ABL protein expressed by CML cells is an appropriate target for T-cell therapy is an open experimental question. In animal models the adoptive transfer of immune tumor antigen-specific $CD4^+$ or $CD8^+$ T cells alone can mediate tumor therapy, but the combination is generally more effective (15). Therapy mediated by immune $CD4^+$ T-cell responses *in vivo* requires that antigen be present in the environment of APCs in high enough concentrations to stimulate the immune T cells to proliferate and to secrete the cytokines involved in direct and/or indirect cytolytic mechanisms. BCR–ABL protein is normally found in the cytosol (35) and is not considered to be a secreted protein, but it might be found in the external cellular environment as a result of normal cell death and turnover or as a result of other therapeutic interventions such as cytolytic chemotherapy. We are not aware of any studies that have examined this issue, but our recent preliminary studies have shown that BCR–ABL protein can be detected *in vitro* in spent culture supernatants. Studies by others have validated that other protooncogene-encoded proteins known to exist normally and function intracellularly can be found in body fluids (36) and in the proximate tumor microenvironment (37). Thus, BCR–ABL protein might be available in the external cell environment at sites of tumor to be processed and presented by autologous APCs expressing class II MHC molecules. The availability of BCR–ABL protein to T cells *in vivo* needs to be evaluated. Irrespective of the amount of BCR–ABL protein that is normally released by CML cells *in vivo*, the demonstration that intracellular protooncogene products are immunogenic and can potentially serve as tumor-specific antigens should focus experimental attention on learning how to target immunity to such antigens.

An alternative approach to focus immunotherapy against BCR–ABL protein would be to develop $CD8^+$ class I MHC-restricted cytolytic responses. The binding of peptides to class I MHC molecules occurs for protein synthesized by the target itself to the exclusion of proteins in the external environment (21). The features associated with high-affinity binding of peptides to MHC molecules are just beginning to be elucidated. However, peptides that bind to class II MHC molecules often also bind to class I MHC molecules (38). Thus, $CD8^+$ class I MHC-restricted cytotoxic T-cell responses to the BCR–ABL joining region segment are conceivable.

The hallmark of malignant transformation is DNA alterations. The immune system normally subserves the function of discriminating self and non-self. Any protein expressed by altered DNA is potentially immunogenic. With knowledge of the DNA sequence of mutated or chimeric cancer-related genes, it should now be possible to derive the amino acid sequences of the aberrant expressed proteins and to determine whether the aberrant proteins are immunogenic to the autochthonous host. The current study confirms the hypothesis that BCR–ABL chimeric protein expressed by Ph^1 in CML is immunogenic by virtue of the unique joining-region amino acid sequences. Although the current study focused on evaluation of immune responses to $p210^{BCR-ABL}$ protein in leukemic cells, it is anticipated that the methods can be extended to the evaluation of the immunogenicity of proteins expressed by other translocated genes commonly associated with leukemia and lymphoma or by somatically mutated cancer-related genes.

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