BCR-ABL rearrangement and HLA antigens: a possible link to leukemia pathogenesis and immunotherapy

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The translocation, t(9;22)(q34;q11), giving rise to the Philadelphia chromosome (Ph), is observed in approximately 30% of acute lymphoblastic leukemia (ALL) and in virtually all chronic myeloid leukemia (CML) patients, where it represents the diagnostic molecular hallmark.

The reciprocal rearrangement joins the 5' sequence of the breakpoint cluster region gene (*BCR*) at 22q11 to the 3' sequence of the c-abl oncogene 1 (*ABL1*) at 9q34, generating the fusion gene *BCR/ABL1*.

Breakpoints in *ABL1* generally involve exon 2 (a2). Breakpoints in *BCR* occur in the major breakpoint cluster region (*M-BCR*) in the great majority of CML patients and in the minor breakpoint cluster region (*m-BCR*) in the bulk of subjects with Ph-positive ALL. Breaks occurring in *M-BCR* juxtapose exon 13 or 14 to *ABL1*, resulting in the fusion transcripts e13a2 (also designated b2a2) and e14a2 (also designated b3a2), respectively. Either *BCR/ABL* RNA messengers translate in a chimeric oncoprotein with molecular weight of 210 kDa (p210 $^{BCR-ABL}$) which harbors constitutive tyrosine kinase activity driving the growth advantage of the leukemic cell clone. Breaks in *m-BCR* bring together *BCR* exon 1 and *ABL1*, yielding a smaller fusion transcript, e1a2, which codes for a 190 kDa chimeric oncoprotein (p190 $^{BCR-ABL}$) with more transforming potential than p210 $^{BCR-ABL}$ [1].

The junctional regions of p210 $^{BCR-ABL}$ and p190 $^{BCR-ABL}$ display a unique amino acid sequence that is not found in any normal protein. Therefore, because the translated BCR-ABL product is expressed only in leukemic cells and not in normal cells, the chimeric peptides encompassing the e13a2, e14a2 or e1a2 breakpoints may act as potential targets for specific T lymphocyte-mediated immune response against CML and ALL tumor cells.

However, T lymphocytes can recognize and can be stimulated to proliferate and to perform the function of leukemia cell killing only if the processed chimeric antigens are assembled at the cell surface in association with major histocompatibility complex (MHC) class I and II molecules.

Several *in vitro* experiments indicate an association between BCR-ABL fusion products and MHC alleles. In particular, different purified class I MHC molecules have been described to bind strongly to peptides spanning the BCR-ABL e14a2 junction, including human leukocyte antigen (HLA) A3 and B8 class I molecules.

Moreover, mass spectrometry studies demonstrated that e14a2 peptides are presented on the cell surface of primary CML cells by HLA-A3 molecules. The results suggest that determined BCR-ABL junctional peptides may preferentially bind to certain HLA alleles thereby supporting the potential of these antigens as targets for class I HLA restricted T lymphocyte cytotoxicity. On the other hand, an efficacious immune response may confer to the individuals carrying these particular HLA alleles an advantage in fighting the leukemia. Indeed, additional *in vitro* studies, in which exogenous cytokines substituting for $CD4^+$ lymphocyte helper function were used, have shown that these BCR-ABL peptides appear to be immunogenic since they can elicit specific class I, HLA-restricted cytotoxic T lymphocyte (CTL) responses in normal donors and CML patients. The findings demonstrate that patients with CML have the *in vitro* capacity to respond to their own individual cancer cells and that CML cells are competent in processing and presenting endogenous immunogenic e14a2 peptides in the context of class I HLA[2].

Although less is known about the association of e14a2 BCR-ABL peptides with HLA class II molecules, support for the immunogenicity of these antigens has been accumulating. It has been demonstrated that it is possible to establish $CD4^+$ T-lymphocyte cell lines restricted for HLA-DRB1*0401 presenting e14a2-derived peptides from healthy subjects and that these cells showed a proliferative response to HLA-DRB1*0401-bearing e14a2-positive CML blasts. On the other hand, these $CD4^+$ T-lymphocyte cell lines did not respond to HLA-DRB1*0401-bearing e14a2-negative cells or HLA-DRB1*0401-negative e14a2-type CML blasts.

In another study, e14a2-derived peptides and HLA-DRB1*0901-restricted $CD4^+$ T-lymphocyte clones were established and their effect on CML cell growth was investigated.

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The number of HLA-DRB1*0901-positive e14a2, but not those of e13a2-positive or HLA-DRB1*0901-negative CML cell colonies appeared to increase when CML cells were cultured with e14a2-specific CD4+ T lymphocyte clones.

The effect of e14a2-specific CD4+ T lymphocyte clones on e14a2-positive CML cell growth was inhibited by the addition of anti-HLA-DR monoclonal antibodies. These data suggest that the BCR-ABL chimeric protein is processed naturally in CML cells and is recognized by BCR-ABL-specific CD4+ T lymphocytes in the context of HLA class II molecules.

To verify this possibility, the ability of dendritic cells (DCs) derived from monocytes of CML patients to present endogenous BCR-ABL chimeric peptides to CD4+ T lymphocytes was investigated. The results showed that CML-derived mature DCs can process and present the endogenous BCR-ABL chimeric protein to BCR-ABL peptide-specific CD4+ T lymphocyte clones in an HLA class II-restricted manner.

However, the sparse available data suggest that CD4+ T lymphocyte responses to BCR-ABL may be hindered in CML patients compared to healthy individuals. Indeed, e14a2 peptides are able to evoke a CD4+ T lymphocyte response in normal subjects, but cannot elicit specific clones from CML peripheral blood.

Much fewer data are obtainable for e13a2 junctional peptides that are shown to bind at low affinity to B8 and A11 MHC class I molecules and to yield T cell proliferative responses in a HLA-DR2a restricted fashion only after repetitive stimulation.

Other scientific studies report analyses of the association between particular HLA alleles and different types of BCR-ABL fusion proteins at a population level, assuming that a negative association of a particular BCR-ABL product with specific HLA alleles suggests that these alleles play a critical role in presenting peptides derived from the chimeric proteins and in eliciting a successful T lymphocyte cytotoxic response[3]. In this perspective, even if it is well known that different populations show different HLA haplotype frequencies, the findings of Carvalho et al.[4], trying to unravel the issue of the association of HLA molecules with BCR-ABL peptides inside the Brazilian population, have the major advantage of raising novel interests about the immune pathogenesis of CML and the CML immune-mediated therapies. In fact, the Carvalho et al. report indicates that BCR-ABL peptides may be presented by different HLA molecules, which inside the specific CML population may elicit a productive (negative association) or ineffective (positive association) binding to leukemic proteins, in comparison with the healthy population. Carvalho et al. showed a positive association between HLA-A25 and HLA-B18 as well as a negative association between HLA-A68 and e13a2 transcripts, whereas they reported a positive association between HLA-B40 and HLA-DRB1*3 with e14a2 transcripts[4]. On the basis of positive/negative associations, it has been assumed that HLA-restricted T lymphocyte cytotoxicity accomplishes an immunosurveillance role in the pathogenesis of BCR-ABL leukemias.

In this regard, beside the aforementioned demonstration of the immunogenicity of the peptides spanning the fusion region of the chimeric proteins presented in the context of MHC class I and II, several other observations provide coincidental evidence for the existence and efficacy of immune reactions in CML patients. For instance, it is well known that BCR-ABL mRNAs have been detected in normal individuals and that both CTL and CD4+ proliferative responses against BCR-ABL can be elicited in normal subjects suggesting the importance of the immune response in controlling and/or eliminating BCR-ABL positive leukemic clones. Even if we do not understand the precise mechanisms of immune escape by the BCR-ABL leukemic clone, causing the clinical emergence of the disease, further proof of an immunologic component in the eradication of leukemia cells comes from the demonstration that CML may respond to immune-mediated therapies, including stem cell transplantation, donor lymphocyte infusion and interferon alpha administration.

This evidence indicates that under *in vivo* circumstances, some, but apparently not always entirely efficient, immune responses against leukemic cells do occur. Hence it may be possible to gain durable remissions by boosting this immunity with vaccination. In animal models, immunization with BCR-ABL specific peptides can raise an antiserum reacting specifically with the native p210 $^{BCR-ABL}$ in CML cell lines and results from small-scale clinical trials using vaccines based on the p210 $^{BCR-ABL}$ chimeric protein obtained beneficial effects in some patients. These findings suggest that immunotherapeutic approaches may supplement the current targeted therapies with tyrosine kinase inhibitors and may be important to attain a definitive cure.

Clinical effects of BCR-ABL peptide vaccination associated with imatinib have already been demonstrated in patients with persistent residual disease and vaccination with BCR-ABL junctional peptides might improve the reduction of *BCR-ABL* mRNAs in patients who had previously received imatinib for more than 12 months.

Analyses of HLA association with different BCR-ABL peptides may have therefore diagnostic and prognostic significance and may advance our knowledge about strategies of BCR-ABL immunization.

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