Immunotherapy with CTLs restricted by nonself MHC

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The experiments that won the Nobel Prize for Doherty and Zinkernagel in 1996 showed that cytotoxic T lymphocytes (CTLs) killed virus-infected target cells only when they expressed the same major histocompatibility complex (MHC) class I molecules as the effector CTLs (Ref. 1). The recently determined crystal structure of T-cell receptors (TCR) bound to peptide-presenting MHC class I molecules provided a molecular explanation for the phenomenon of MHC-restricted T-cell recognition2,3. Particularly informative was the crystal structure of a human TCR specific for a 9-residue peptide presented by HLA-A0201 class I molecules3. In total, this TCR made contact with 23 residues, 7 contacts involving the peptide, 6 the α1 helix and 10 the α2 helix of the HLA molecule.

Thus, in molecular terms, MHC restriction was accounted for by 16 contact points between the TCR and the α1 and α2 helix of the A0201 molecule. Only 5 of these 16 TCR contact residues were polymorphic, while the remaining 11 were conserved in different HLA alleles. A sequence comparison of 75 HLA-A locus alleles revealed that the 16 TCR contact residues were identical in 30 alleles, 35 alleles showed a difference in 1 residue, 9 alleles in 2 residues and 1 allele in 3 residues. This demonstrated that the residues contacted by an HLA-A0201-restricted TCR were conserved in approximately 30% of allogeneic HLA-A locus alleles. Based on these observations it is predicted that recognition of specific peptides presented by allogeneic MHC molecules with conserved TCR interaction sites might be as efficient as recognition of peptides presented by self-MHC molecules.

Allo-MHC-restricted cytotoxic T lymphocytes can be directed against peptides that are preferentially expressed in malignant cells. Here, Hans Stauss discusses how these cells can mediate a graft-versus-leukaemia reaction without causing graft-versus-host disease when used for immunotherapy in bone-marrow-transplanted leukaemia patients.

Allo-MHC-restricted CTLs against tumour-associated peptides

The concept of specific peptide recognition in the context of allogeneic MHC molecules provides a unique opportunity to guide T-cell responses in bone-marrow transplantation. Patients suffering from leukaemia frequently receive bone-marrow transplants from donors who, despite serological HLA matching, are genetically mismatched. It is well established that donor lymphocytes can mount immune responses against recipient leukaemic cells resulting in a graft-versus-leukaemia (GVL) reaction, which is highly desirable because it significantly improves disease-free survival4,5. Unfortunately, the GVL effect is often associated with a graft-versus-host disease (GVHD), whereby donor lymphocytes attack and destroy normal host tissues6. This indicates that donor lymphocytes recognize determinants present on both leukaemic and normal cells. We have started to investigate the possibility of dissociating the beneficial GVL reaction from the detrimental GVHD by
directing the immune response of donor lymphocytes against peptide determinants that are preferentially or exclusively expressed by leukaemic cells.

### Murine allo-MHC-restricted CTLs

The idea of raising CTLs against peptides expressed by MHC-mismatched lymphoma cells has been explored in a murine model. CTLs were isolated from BALB/c mice (H2b haplotype) against a selected peptide expressed in RMA lymphoma cells of H2b haplotype. The peptide was derived from the cellular protein mdm-2, which has transforming activity and is often overexpressed in tumour cells. Initially, the peptide was identified by screening the mdm-2 protein sequence for H2Kb class I binding motifs, and by confirming in binding assays that the selected peptide indeed bound to Kb. The transporter associated with antigen processing (TAP)−negative cell line RMA-S (H2b) was used to generate peptide-specific CTLs from BALB/c mice.

A substantial proportion of Kb class I molecules expressed by RMA-S cells are ‘empty’ and can be loaded with exogenously added synthetic mdm-2 peptides. When these peptide-loaded cells were cocultured in vitro with naive BALB/c lymphocytes, they efficiently stimulated CTL clones specific for the selected peptide presented by the allogeneic Kb class I molecules. These allorestricted CTLs lysed the RMA lymphoma cells that expressed mdm-2 endogenously, while the level of mdm-2 expression in non-transformed cells was insufficient to trigger CTL killing. Thus, although the CTLs were directed against a widely expressed cellular protein, they were functionally tumour-reactive because the level of peptide produced in normal cells did not reach the threshold required to elicit CTL effector function.

As expected, H2b mice were tolerant because they contained only low avidity CTLs against this Kb-presented peptide epitope. These CTLs were unable to recognize transformed cells expressing mdm-2 endogenously. This illustrated the inability of autologous CTLs to mount immune responses against the tumour-associated mdm-2 protein; this inability was overcome by using CTLs from MHC-mismatched donors.

The diversity of the allo-MHC-restricted T-cell repertoire has been investigated in a recent study. BALB/c responder cells were stimulated with a synthetic peptide library binding to Kb. The results showed that the allorestricted T-cell repertoire is highly diverse, indicating that it should be possible to direct CTLs against a large variety of tumour-associated peptide epitopes.

Allorestricted CTL clones specific for an mdm-2-derived epitope inhibited tumour growth in vivo when they were co-injected with H2b-derived RMA lymphoma cells into H2b recipient mice. However, tumour protection in immunocompetent mice was limited to a time period of approximately two weeks, suggesting that recipient mice mounted an immune response against H2b-derived CTLs leading to their elimination and a breakdown of tumour protection. This was confirmed in experiments using H2b × H2b F1 mice expected to be unable to mount immune responses against H2b-derived CTLs. When these mice were injected with RMA lymphoma cells and allorestricted CTLs, long-term tumour protection was observed (H. Stauss, unpublished).

These results indicate that efficient tumour protection is dependent upon long-term survival of allorestricted CTLs in the recipient host. Although F1 recipients can be conveniently employed in murine experiments to avoid rejection of injected CTLs, this will not be possible in humans where allorestricted CTLs will be adoptively transferred into MHC-mismatched recipients. As discussed below, recent experiments indicate that long-term survival of allorestricted CTLs can be achieved in MHC-mismatched recipients undergoing bone-marrow transplantation.

### Human allo-MHC-restricted CTLs

The concept of generating allorestricted CTLs specific for peptides presented by non-self MHC molecules has been investigated in humans. The diversity of the allo-MHC-restricted T-cell repertoire has been explored in a recent study. BALB/c responder cells were allostimulated with a synthetic peptide library binding to HLA-A2. These experiments establish that it is possible to exploit the T-cell repertoire of donors to raise allo-MHC-restricted CTLs specific for HLA-A2-presented peptide epitopes expressed at elevated levels in tumour cells. These findings support the possibility of raising allo-MHC-restricted CTLs specific for leukaemia-associated peptide epitopes.

### Adoptive immunotherapy

Selection of targets for leukaemia-reactive CTLs

There are a number of transcription factors and differentiation antigens expressed exclusively in haematopoietic cells. In particular, the transcription factors WT-1 and GATA1, and the differentiation antigens myeloperoxidase and CD68 have been shown to be expressed in leukaemic cells; these therefore provide potential targets for leukaemia-reactive CTLs. However, because these proteins are expressed in normal haematopoietic cells, it is likely that they establish tolerance. Consequently, autologous T cells are unlikely to mount CTL responses against leukaemic cells with elevated levels of...
protein expression. By contrast, the allo-MHC-restricted T-cell repertoire is ideally suited to isolate CTLs specific for haematopoietic transcription factors and differentiation antigens. As the selected target proteins are not expressed in non-haematopoietic tissues, adoptive immunotherapy with allorestricted CTLs would be expected to result in the elimination of leukaemic cells without causing GVHD.

**Human model**

The aim is to establish CTL clones from HLA-A2 individuals specific for HLA-A2-presented peptides derived from haematopoietic proteins. Ideally, a single CTL clone would be used for adoptive immunotherapy of numerous HLA-A2 leukaemia patients, irrespective of the degree of MHC mismatch between CTL clone and patient. This would overcome the current need to generate customized CTL clones for each patient. Indeed, murine experiments described below indicate that successful CTL engraftment is not dependent upon MHC matching between injected CTLs and the recipient host. Adjuvant immunotherapy can be combined with allogeneic bone-marrow transplantation, deliberately selecting HLA-A2 donors (Fig. 1a). Thus, allo-MHC restricted CTLs specific for A2 presented peptides will attack leukaemic cells and perhaps some normal haematopoietic cells of recipient origin if they express sufficient levels of the relevant target protein, but A2 haematopoietic cells of donor origin will be completely resistant to CTL attacks thereby allowing efficient bone-marrow engraftment.

The suggested immunotherapy with allorestricted CTLs is dependent upon CTL survival in MHC-mismatched hosts. Recent experiments in humans have shown that immunocompetent recipients can mount specific CTL responses against adoptively transferred CTL (Ref. 13). However, it is likely that the severe immunosuppression required for successful bone-marrow transplantation will provide a unique window of opportunity for engraftment of MHC-mismatched CTL clones. Thus, it is predicted that patients who are conditioned with irradiation and immunosuppressive drugs before bone-marrow transplantation, deliberately selecting HLA-A2 donors, can at the same time accept MHC-mismatched CTL clones. Furthermore, CTL clones administered during a period of immunosuppression might induce specific tolerance as patients regain immunocompetence. These predictions have been supported by experiments in a murine model (Fig. 1b).

**Murine model**

In this model (C57BL/6 recipients (H2b) were transplanted with bone-marrow from MHC-mismatched B10.A(4R) donors. The MHC mismatch was selected to include the H2K locus, with recipients expressing the Kb and donors the Kk allele. Mice were conditioned with 1000 Rad whole body irradiation. They were then injected with T-cell depleted bone-marrow and CTL clones isolated from third party BALB/c mice (H2d). This model was designed to test whether allorestricted CTLs would survive and function in fully MHC-mismatched hosts, as this situation might occur in adoptive immunotherapy of humans (see above). The BALB/c-derived CTLs were specific for an mdm-2 peptide presented by Kk class I molecules. The CTLs recognized Kk recipient mouse bone-marrow CD45RA+ lymphoid tissues of CTL clones in long-term survival of individual patient. CTLs are injected at the same time as bone-marrow cells to exploit the transient immunosuppression required for successful bone-marrow transplantation. Bone-marrow donors and recipients are HLA-matched except for the peptide-presenting HLA allele. Consequently, only recipient bone-marrow cells, but not donor cells are susceptible to CTL attack. Because expression of the selected CTL-recognized peptide is limited to the haematopoietic lineage, CTL attack of non-haematopoietic tissues and GVHD is unlikely to occur. (b) Murine model to test the feasibility of adoptive immunotherapy with a third-party allorestricted CTL clone isolated from BALB/c mice and specific for a peptide derived from the mdm-2 protein presented by Kk class I molecules. Kk-expressing C57BL/6 mice were transplanted with bone-marrow from Kk+ B10.A(4R) mice and injected with allo-MHC-restricted CTL. Recipient mice did not develop GVHD. CTLs were detectable in lymphoid tissues of recipient mice 3 months after injection, indicating successful CTL engraftment into fully MHC-mismatched hosts. Abbreviations: CTL, cytotoxic T lymphocyte; GVHD, graft-versus-host disease; MHC, major histocompatibility complex.
cells provided they expressed sufficient amounts of mdm-2, but were unable to recognize K\(^+\) bone-marrow donor cells.

Several weeks after injection the CTLs were present in the spleen, mesenteric and peripheral lymph nodes and in the thymus. In vitro analysis of recovered CTLs showed that they maintained their specificity for the mdm-2 peptide presented by K\(^+\) class I molecules. L. Gao et al., unpublished. Experiments performed to date indicated that BALB/c-derived CTLs were detectable 3 months after injection into C57BL/6 recipients. Thus, complete MHC mismatch between CTLs and the recipient host is not a barrier for long-term CTL survival. As the H2\(^+\)-derived CTLs were present in the thymus of recipient mice it is possible that they induced tolerance to H2\(^+\) allogens. This possibility needs to be tested in detail.

It is important to note that the allo-MHC-restricted CTL did not cause GVHD although the capacity to execute peptide-specific effector function was unimpaired. This suggests that transferred CTLs have the potential to provide long-term protection against transformed cells expressing sufficient levels of the relevant target protein to trigger CTL killing.

**Conclusion**

Allo-MHC-restricted CTLs are promising reagents for adoptive immunotherapy of bone-marrow-transplanted leukemia patients. They can be raised specifically against peptides that are preferentially or exclusively expressed in leukemic cells. It is becoming clear that the peptide specificity is not the only factor that determines selectivity of target cell killing by CTLs. Although CTLs can be directed against cellular proteins expressed in normal cells, target cell killing is often specific for transformed cells displaying a certain threshold level of peptide required to trigger CTL effector function. Deliberate MHC mismatches can be exploited to further improve the selectivity of allorestricted CTL by choosing bone-marrow donors who do not express the MHC alleles required for peptide presentation.

One of the main attractions is that allorestricted CTLs can be raised against any cellular protein known to be expressed at elevated levels in tumours. This provides an experimental framework for the new area of tumour immunology, where CTL-recognized target antigens are selected based on information concerning abnormal protein expression in tumours. Transcription factors are particularly interesting because upregulated expression is frequently associated with transformation. Recently, murine CTLs specific for the p53 transcription factor were shown to protect against growth of tumours overexpressing this protein. Tolerance was circumvented by isolating CTLs from p53-deficient gene-knockout mice. In the human situation, it will be possible to circumvent tolerance by the use of allorestricted CTLs.

The allorestricted CTL repertoire can provide tumour-reactive specificities that cannot be found in the autologous T-cell repertoire. In the future, it will be feasible to develop novel protocols for gene therapy of tumours by cloning the TCR chains from allorestricted CTLs, introducing them into autologous CTLs and thereby equipping them with a tumour-reactive specificity that they do not naturally possess.

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**References**


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