Introduction

Initially, the treatment of haematological malignancies predominantly made use of the antimetabolites (methotrexate, hydroxyurea) or alkylating agents (cyclophosphamide, busulphan) that achieved a normalization of the white blood cells count but were associated with serious side effects (such as marrow aplasia).

The introduction of α-interferon (IFN-α) in the early 1980s contributed to an increase of overall survival compared to chemotherapy of about 20 months. Furthermore, the complete response in some patients treated in chronic phase was ascribed to its immunomodulatory effect, i.e., upregulation of HLA class I and II and of adhesion molecules.

Allogeneic haematopoietic stem cell transplantation (HSCT), in addition to the diminution and eradication of some of the tumour burden by high dose pretransplant chemo and radiotherapy regimens, provides an allogeneic antitumour effect or graft-versus-leukaemia (GVL) effect and truly constitutes the only current curative treatment for chronic myeloid leukemia (CML). Thus, the major curative effect of allogeneic HSCT is due to immune responses directed against malignant cells, either targeting specific tumour markers (antigens) or, more likely, individual-to-individual antigenic differences between the donor and recipient.

Donor lymphocyte infusion (DLI) has been shown to cure 20–80% of the patients with relapsed leukaemia or lymphoma depending on the type and extent of the disease. However, graft-versus-host disease (GVHD) often accompanies treatment using unmodified DLI. In vitro selection and expansion of cytotoxic T lymphocytes (CTL) with relative specificity for the malignant cells may separate the anti leukemic responses from GVHD. Although “proof of principle” for this approach has been demonstrated in a number of patients by successful eradication of the relapsed haematological malignancy by infusion of leukaemia reactive CTL, therapeutic numbers of T-cells with the desired antigen specificities are difficult to obtain and process in vitro. Moreover, with a very small number of exceptions, such as the minor Histocompatibility antigens HA-1 and HA-2, the differences between individuals that are capable of giving rise to these beneficial immune antitumour responses and not to a detrimental anti-host reaction, GVHD, have not been characterized. In consequence, this potentially immunotherapeutic effect of allogeneic transplantation has so far been an untargeted and unpredictable property of stem cell transplantation (SCT).

Suitable targets for tumour immunotherapy fall into a number of discrete classes.

- A tumour-specific or tumour-associated peptide presented by either allo or self-MHC.
- A polymorphic lineage restricted peptide presented by self-MHC.
- A monomorphic lineage restricted peptide presented by allo-MHC.

The ideal targets are novel molecules specific to the tumour and are derived from genetic modifications that arise in the tumour cell or its progenitor. For example, chronic myeloid leukaemia CML arises as a result of a chromosomal translocation event that activates the ABL tyrosine kinase. In the translocation process, two genes – the BCR (breakpoint chromosomal region) and ABL - are fused, creating a novel fusion protein, BCR/ABL. The sequence of the junction between the BCR and ABL portions is unique to the tumour cells and is not expressed in any normal cell of the body; immune responses directed against this fusion peptide could eliminate the tumour cells while normal cells are unaffected.
In order to identified leukaemia-specific targets, we at the Anthony Nolan Research Institute have used K562, a Ph+ cell line, which expresses b3a2 BCR-ABL mRNA. These cells were transfected with single HLA-A*03011 or HLA-B*08011 alleles by electroporation. Acid elution of transfected K562 transfectants as well as from HLA-A3 or HLA-B8 B*08011 alleles by electroporation. The resultant peptides were analysed by mass spectrometry with nanospray ionisation. Sequencing results obtained confirmed the presence of the HLA-A*03011 restricted peptide KQSSKALQR on both transfected K562 and primary CML cells. Tetramers of HLA-A3 and HLA-B8 with the corresponding eluted peptides were produced. These are currently being used to detect antigen-specific CTLs from HLA-A3 and HLA-B8 patients at various stages of their disease and treatment.

Additionally we have been able to demonstrate that these cells can be expanded in vitro. These in vitro expanded cells demonstrate cytotoxicity for autologous leukaemic cells but not for third party HLA disparate cells. It has also been possible to demonstrate the presence of BCR/ABL reactive cells in the circulation of CML patients pre-grafting.

A phase 1 peptide vaccination study has now been initiated with our clinical collaborators, utilising a combination of the peptides defined for HLA-A3 and HLA-B8 together with a pan class II peptide in an attempt to induce any existing memory response and also to provide class II help for the emerging class I antipeptide response. The vaccinations will be administered in patients where the tumor burden has been reduced hopefully allowing the patients own endogenous antitumour response to emerge.

We have also been investigating routes for the efficient expansion of antigen specific T-cell populations and the development of delivery systems for in vivo stimulation and expansion of specific T-cell populations. These data demonstrate that [1] CML cells do express HLA-associated leukaemia-specific peptides and that [2] CML patients have circulating CTLs specific for the b3a2 fusion peptides. [3] That these cells can be expanded with specific peptides in vitro, and also that these cells are capable of demonstrating specific cytotoxicity for autologous leukaemic cells in vitro. These findings provide encouragement for an eventual immunotherapeutic approach for the treatment of CML by in vitro stimulation of patients T-cells against specific leukaemic peptides providing the basis for immunotherapeutic intervention in CML.

The future of Immunotherapy for the treatment of life-threatening opportunistic infections after HSCT

Following allogeneic HSCT, infections remain of major concern in the management of patients. Due to improved post-transplant antimicrobial prophylaxis and early intervention with broad-spectrum anti-infectives the main problems with infectious complications occurs in the post-transplant period after neutrophil engraftment. During this time period, herpes virus infections as well as invasive fungal infections (predominantly caused by Aspergillus species) are associated with significant morbidity and mortality. In spite of new antiviral and antifungal agents and new diagnostic and therapeutic strategies cytomegalovirus (CMV) and EBV-associated clinical manifestations as well as invasive Aspergillosis are associated with a mortality of >70% in recipients of an allogeneic stem cell transplant recipient. Additionally in certain subgroups of patients (recipients of a haploidentical graft, of an extensively TCD graft) Adenovirus, varicella zoster virus (VZV) and BK virus infection cause significant morbidity and mortality. Adoptive immunotherapy for these infectious complications, to deliver specific protective immunity is therefore highly desirable.

Cytomegalovirus and HSCT

The careful monitoring of SCT patients facilitates the rapid detection of an infection and its subsequent treatment with broad-spectrum antibiotics. CMV is a particular problem post HSCT and can cause life-threatening pneumonitis in patients who reactivate or acquire CMV. Approximately 40–60% of the developed world’s population are infected with CMV. In healthy individuals the virus remains dormant and is effectively maintained in this latent state by CMV specific CD8+ T lymphocytes. The resolution of a CMV infection is followed by the emergence of a memory subset of these cells to deal with any future reactivation events. In the HSCT setting since the recipients immune reconstitution is delayed and the patient is slow to develop CD8+ T lymphocytes CMV occurrence in SCT patients is common. There are predisposing factors that increase the chances of developing CMV infections such as a CMV positive donor and recipient, a matched unrelated donor and the length of immunosuppressive therapy. The monitoring of patients CMV viral load can help clinicians to detect early signs of CMV reactivation allowing for the prompt treatment with antiviral therapy.

Adoptive therapy

The adoptive transfer of CMV specific CD8+ T lymphocytes is a potential alternative treatment for patients at high risk of developing CMV disease and who are not responding to antiviral therapy.

The identification of the CMV epitopes that are presented by the infected cells would allow the generated CTL populations to be boosted by the use of a peptide vaccine. Early work carried out by us...
at the Anthony Nolan Research Institute had identified the pp65 protein as the main target antigen in CMV positive individuals, this protein is a good vaccine candidate as its presentation on infected cells is independent of viral genome expression. The CMV peptide NLVPMVATV was identified as being present in HLA-A*0201 positive individuals.

CMV tetramers

The arrival of tetramers has opened up new possibilities for the use of the peptide epitopes once identified. The production of CMV class I tetramers has enabled the monitoring of patients post HSCT such information can be used to guide the clinicians with regard to antiviral therapy and also to monitor patients who have undergone CMV immunotherapy. It has been shown that CMV specific CD8+ T-cells can be detected shortly after the transplant and the maintenance of these levels protects the patient from subsequent reactivation. Work from our own experience in HSCT patients who experienced a drop in the number of CD8+CMV-specific T-cells their risk of developing CMV disease was highly increased and this risk was also associated with those patients on prolonged immune suppression or those who experienced GVHD. As HLA class I tetramers have also enabled us the monitoring of patients post HSCT and to use their information to guide clinicians on the treatment of patients and whether intervention with adoptive therapy is necessary.

Finally, adoptive transfer of pathogen-specific T-cells can be combined with vaccination strategies and present a platform of tuning the cellular response in vivo for the control of CMV and other infections.

Conclusion

We, at the Anthony Nolan Research Institute have focussed on methods to improve the outcome of HSCT by improving the degree of matching and by implementing immunotherapy. Regarding HLA, we have found that the use of a high-resolution tissue-typing technique is crucial to identifying allelic mismatches and therefore should be the method of choice for typing pre-transplantation. Mismatching for class I and class II alleles predicts for a worse transplant outcome.

In addition, we feel that the identification of the target antigens and effector cells of the GVL response may allow for the separation of GvHD from GVL and could result in novel treatment strategies which could substantially improve transplant outcome.

Finally, the characterisation of conserved CMV peptide epitopes plays an important role in providing valuable information for the design of vaccination strategies and in the production of reagents for the monitoring of antiviral responses in transplanted patient.

References


