Immunological weapons against acute myeloid leukaemia

JOANNA GALEA-LAURI *Department of Molecular Medicine, GKT, School of Medicine, The Rayne Institute, London, UK*

SUMMARY

A better understanding of the biology of malignant cells and of the host immune system together with dramatic advances in technology have led to the design of innovative immune-mediated approaches to control neoplastic clones, including various haematological malignancies. One of the major problems with conventional cancer therapies is their inability to eradicate residual cancer cells that are resistant to therapy, hence immune intervention might improve the clinical outcome of patients. This mini-review will focus mainly on immunological approaches to the therapy of acute myeloid leukaemia (AML), a subset of a much larger family of leukaemias. Immune-mediated approaches ranging from allogeneic lymphocyte transplants to cytokine therapy, immune-gene therapy and vaccination by dendritic-cell-based vaccines will be discussed.

BACKGROUND TO AML

AML is a neoplastic disorder characterized by the clonal expansion of non-lymphoid haemopoietic progenitor cells resulting in failure of normal haemopoiesis.[1] AML is heterogeneous at morphological, biological and molecular levels. Attempts have been made to classify the different subtypes of AML based on the morphological and cytochemical criteria of the French–American–British (FAB) classification system.[2] Molecular genetic abnormalities consistently associated with distinct forms of AML most probably now confer the most important prognostic information.[3] Current intensive combination chemotherapy protocols achieve complete remission in over 80% of patients but although some patients will be cured, the majority will relapse even after consolidating courses of therapy. Allogeneic or autologous bone marrow transplantation (BMT) has been employed as a means of further intensifying the doses of chemotherapy and radiotherapy. Allogeneic BMT offers the advantage of uncontaminated marrow but involves a high risk of immunological reactions between donor and recipient (graft-versus-host disease; GVHD), as well as between recipient and donor (graft rejection). GVHD is a major cause of morbidity and mortality. In addition, the toxicity of the procedure and the need for a human leucocyte antigen (HLA) -compatible donor limits the availability to less than 10% of AML patients. Using autologous bone marrow during remission avoids most of the immunological problems but has the risk of returning contaminating leukaemic cells to the patient. Despite the use of intensive chemotherapy and BMT, only about 15% of all AML patients will remain alive 5 years after diagnosis,[4] with a slight improvement over the last few years (http://www.lrf.org.uk). Thus the challenge in treating AML is not in inducing remission after diagnosis but lies with the prevention of relapse, i.e. eradication of minimal residual disease (MRD), and this is where the hope of immunotherapy lies for this disease.[5–7]

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This review is dedicated to the memory of Dr William J. R. Hirst, who sadly lost his battle against cancer in November 2000. Will was a key member of the department of Haematological Medicine and The Immune Gene Therapy Programme at King's College Hospital, London. His research work focused mainly on mechanisms leading to immune tolerance in leukaemia. He is greatly missed by all who knew him and especially by those who had the privilege of working closely with him.

Correspondence: J. Galea-Lauri, Department of Molecular Medicine, GKT, School of Medicine, The Rayne Institute, 123, Coldharbour Lane, London SE5 9NU, UK. E-mail: joanna.galea-lauri@kcl.ac.uk

TUMOUR ANTIGENS ASSOCIATED WITH AML

Immense optimism for cancer immunotherapy has been attributed to the discovery of several tumour antigens and the characterization of antigen-specific T cells at a single-cell level.[8] Of course, the real key to successful immunotherapy is to identify which antigens should be targeted therapeutically. To this end, the term 'tumour antigen' cannot be used as a synonym for 'tumour-rejection antigen' or

'tumour regression antigen'. Clearly, not all tumour antigens identified can induce immune responses leading to tumour rejection. An important lesson comes from the MART-1/Melan A antigen in melanoma patients, whereby despite the detection of high numbers of antigen-specific T cells by use of MART-1/major histocompatibility complex-specific tetramers, attempts to boost the immune response to this antigen by different vaccination approaches have been disappointing.[9] This is probably a result of the loss of this specific antigen during progression of the disease. So what makes a tumour antigen a tumour rejection antigen? As described by Gilboa 'tumour rejection antigen is an operational term describing how well an immune response elicited against a tumour antigen will impact on the tumour growth'.[10] This of course depends on the nature of the antigen and on the immune response to the antigen. Thus an ideal tumour rejection antigen would need to elicit high-avidity T-cell responses and recruit a large number of T cells with considerable diversity in T-cell receptor usage. Thus, it is unlikely that tumour antigens that are also self-antigens will be tumour rejection antigens as tolerance would limit the number of high avidity T cells, thereby decreasing frequency and diversity. The most likely candidates for tumour rejection antigens are probably either neo-antigens, e.g. based on missense mutations or chromosome translocations giving rise to novel peptide sequences within the expressed protein, or antigens that are ignored by the immune system.

AML should be ideal for immunotherapy as several chromosome abnormalities, mainly translocations, have been described in 50–90% of cases.[11] For example, two of the well-characterized AML abnormalities are chromosome translocation 15/17 and translocation 8/21 which result in chimeric gene products PML/RARα and ETO/AML1, respectively.[12,13] Missense point mutations for RAS and TP53, and mutations in FLT3 because of internal tandem duplication, have also been described for specific AML subtypes and are commonly found in several elderly AML patients.[14] Some of these abnormalities might serve as important molecular markers to predict the prognosis of patients with AML, as suggested in a study of over 200 newly diagnosed patients with *de novo* AML.[15]

Normal proteins over-expressed in leukaemia progenitors may also provide alternative targets for therapy. Ideally, such antigens should be expressed at substantially higher levels in leukaemic cells and be efficiently processed and presented by diverse HLA class I molecules. Two proteins that fit this description are proteinase 3 (PR3)[16] and Wilms' tumour-suppressor (WT1)[17] both of which have already been shown to elicit cytotoxic T-cell responses. For WT1, two different peptides have been described, both of which are restricted to HLA-A24 and used for the generation of cytotoxic T-lymphocyte (CTL) lines capable of lysing HLA-A24 leukaemia lines.[18] MUC1, an epithelial mucin over-expressed in many epithelial malignancies, has also been shown to be expressed on AML blasts, and to be capable of eliciting CTL responses.[19] Another more recently discovered antigen is one of the cancer testis antigens named HAGE, found to be expressed in 23% of AML (Barbara Guinn, in press).

HAEMOPOIETIC CELL TRANSPLANTATION AS IMMUNOTHERAPY FOR AML

Haematological cell transplantation (HCT), originally used to allow higher dose systemic chemotherapy or chemo-radiotherapy, represents the clearest example of the power of the human immune system to eradicate cancer.[20] The initial rationale for HCT came from laboratory and clinical observations that most haematological malignancies exhibit a steep dose–response reaction to alkylating agents and radiation therapy. Because marrow toxicity is dose limiting for many of these agents, by transplanting pluripotent haemopoietic stem cells (bone marrow or from peripheral blood) it became possible to pre-administer higher doses of therapy than was otherwise possible. It is now known that immunocompetent cells transplanted with the stem cells, or arising from them, exert a potent graft-versus-leukaemia (GVL) effect independent of the effects of the high-dose chemotherapy. Barnes *et al.* first demonstrated the existence of GVL as early as 1956, when they reported eradication of leukaemia in irradiated mice receiving allogeneic as opposed to syngeneic marrow transplants.[21] Early evidence for GVL in humans came from studies reporting that relapse rates following allogeneic BMT were markedly less in patients who developed both acute and chronic GVHD compared to those who did not.[22] Later studies also showed that relapse rates were highest if T cells were depleted from the marrow graft[23] or in recipients of twin transplants.[24] More evidence of the immunological efficacy of allografts against leukaemia comes from donor lymphocyte infusions (DLI) which have been successfully used to induce remission in AML patients who have relapsed after an allograft.[25] The key to successful HCT and DLI is to control the occurrence and toxicity associated with acute and chronic GVH disease. In this regard, the possibility of generating anti-leukaemic restricted T-cell clones with specific activity for AML blasts is proving an exciting area of research.[26]

The immunological reactions accompanying allogeneic HCT have been attributed to the HLA system, with the most influential genes being HLA-A, HLA-B and HLA-C (collectively referred to as class I) and DRB1, DQB1 and DPB1 (collectively referred to as class II). These genes are highly polymorphic, e.g. more than 125 HLA-A, 260 HLA-B, 75 HLA-C have been described. The HLA molecules themselves are termed major histocompatibility antigens and T cells confronting non-self HLA molecules react vigorously. The peptides presented by HLA molecules are mostly derived from endogenous proteins, including peptides from the HLA molecules themselves. However in the context of transplantation, polymorphisms in these endogenous proteins serve as sources of minor histocompatibility antigens and form the basis of immunological non-identity between HLA-matched individuals.

CYTOKINE THERAPY FOR AML

It is no great surprise that one of the most obvious cytokines to concentrate on for immunotherapy has been interelukin-2 (IL-2).[27] Its pleiotropic effects and expression of its receptor on multiple cell types have been recognized for several years.[28] The use of IL-2 in the management of haematological malignancies has already been reviewed elsewhere.[29] Not only does IL-2 play a critical role in the activation and proliferation of T cells but can generate lymphokine-activated killer (LAK) cells capable of lysing several human neoplastic cells including autologous leukaemic blasts, otherwise resistant to natural killer (NK) effectors.[30] Pre-clinical studies have shown that IL-2 with or without LAK cells may eradicate murine leukaemia.[31] The first attempts to use IL-2 in the management of patients with acute leukaemia date back to the late 1980s.[32] While virtually no responses have been observed in patients with leukaemia crises, responses to IL-2 have been reported in patients with relapsed or refractory AML. In a proportion of patients with residual disease it has been shown that repeated 5-day cycles of high-dose recombinant IL-2 (intravenous infusion) may induce long-lasting complete remissions. More encouraging are the results from a trial in adult patients with AML in first remission suggesting that this group of patients may benefit from IL-2 maintenance therapy.[33]

Although the IL-2 trials are encouraging, unpredictable haematological responses and toxicity associated with high doses have limited the clinical application of IL-2 therapy. However, to overcome such problems combined cytokine therapy is being evaluated, such as the use of IL-2 with IL-12. Several biological advantages of IL-12 include induction of interferon-γ (IFN-γ) and tumour necrosis factor-α by NK and T cells, activation of NK cells, specific CTL responses and initiation of cell-mediated immunity via polarization and regulation of T helper type 1 (Th1) and Th2 T cells.[34] Results on the susceptibility of AML samples to the lytic activity of both allogeneic and autologous peripheral blood lymphocytes stimulated with different combinations and concentrations of IL-2 and IL-12 have shown two remarkable effects: first, combined IL-12 and IL-2 induced lysis of blasts resistant to classic LAK effects; and second, when used in combination with IL-12, the doses of IL-2 needed to achieve the same degree of lysis to that obtained by high-dose IL-2, were very much reduced.[35] Another cytokine worth investigating is IFN-α, although its use in AML has been somewhat limited. In a case study, a poor-risk AML patient commenced IFN-α treatment (Roferon) in second chemotherapy-induced remission phase and remained in complete remission for up to 2 years during the follow-up study.[36]

CANCER IMMUNE-GENE THERAPY AND ITS APPLICATION TO AML

The development of clinically applicable gene transfer systems has opened up a new therapeutic arena for the treatment of malignant diseases including leukaemia. Many different strategies are being explored to exploit this technology: gene-marking studies, drug sensitivity genes, drug-resistance genes, targeting of oncogenes or tumour-suppressor genes and, more relevant to this review, gene-modified immunotherapy. One of the major advantages of immune-gene therapy is the preclinical observation of a bystander effect suggesting that not every cell needs to be transduced. Several animal models have shown that the tumorigenicity of many cell lines can be reduced by the expression by the tumour of immunomodulatory genes such as cytokines, immune co-stimulators and even HLA molecules.[37,38] The advantages of IL-2 cytokine therapy have been highlighted above, however, in view of the limitations and toxicity associated with the exogenous administration of IL-2, efforts have focused on the transduction of the IL-2 gene. The successful insertion of the IL-2 gene by a retroviral vector into human leukaemic cell lines of both myeloid and lymphoid origin was reported in 1994, and shown to reduce or abrogate the *in vivo* tumorigenic potential in T-cell-deficient nude mice.[39] IL-12 has also been transduced into murine AML blasts. In contrast to systemic IL-12 administration, vaccines with irradiated IL-12 AML cells can cure mice bearing a considerable leukaemic burden and can protect naïve mice against challenge with wild-type AML cells.[40]

Another strategy to enhance anti-leukaemia immunity has been to introduce the B7.1 gene into the leukaemia cells as a means of expressing one of the major co-stimulatory molecules required for T-cell activation upon its interaction with CD28 on the T-cell surface. Details of the B7 family of ligands and its receptors could be found in the latest annual reviews of immunology.[41] Initially it was thought that the introduction of B7.1 into tumour cells would allow the modified tumour cells to act as antigen-presenting cells (APC) directly activating the T cells. However, subsequent studies have shown that bone-marrow-derived APC play a key role in the anti-tumour response.[42] It is possible that B7.1 provides a signal for NK activation and induces NK-mediated tumour lysis,[43] thus shedding tumour antigens that are taken up by APC to be presented to T cells by cross-presentation.[42] The first report on the role of B7.1 in a murine leukaemia model showed that a single exposure to live non-irradiated B7.1 genetically modified leukaemic cells induced protection against subsequent challenge with B7.1 negative leukaemia cells. Furthermore, hyperimmunization with B7.1-modified leukaemia cells prolonged the survival of mice previously injected with a lethal number of unmodified leukaemia cells.[44] Two years later a different laboratory published similar results in a murine AML model using irradiated B7.1-modified AML blasts, as a prophylactic vaccine and moreover showed that in a treatment model rejection of leukaemia was only observed if vaccination took place early in the disease.[45] Later, the same group published other studies on an even more potent anti-leukaemia protective and therapeutic immunity using either GM-CSF- or IL-12-modified leukaemia cells.[40,46] The first report for human B7·1-modified AML blasts showed encouraging T-cell responses, albeit in an allogeneic setting.[47] In later studies, other groups have also shown that

B7.1 can modulate the immunogenicity of other leukaemia cells[48] and induces the generation of leukaemia reactive $CD4^+$ T cells from HLA-identical donors.[49]

Two alternative strategies for gene therapy for the surface expression of B7.1 on AML blasts have been described. In the first study, we have shown that activating antibodies to the co-stimulatory receptor CD28 could be attached to the surface of AML cells via a biotin–avidin bridge, and that such modified tumour cells induce T-cell-proliferative responses.[50] This method only requires off-the-shelf reagents, is quick, cheap and easy to perform and most importantly induces T-cell responses. The other method takes advantage of the surface expression of CD64 (high-affinity Fc receptor) on the leukaemia cells and uses a soluble B7.1 immunoglobulin G (IgG) fusion protein.[51] Targeting of B7.1 IgG to the AML cells resulted in increased proliferation of autologous remission T cells and had the potential to generate an enhanced redirected cytotoxic T-cell response against autologous AML blasts.[51]

COMBINATION GENE THERAPY

Given the complexity of the immune responses involved, investigators are also developing means to introduce multiple genes simultaneously into the tumour cells, allowing the co-ordinated expression of two or more genes. Several preclinical studies documenting the efficacy of combining B7.1 with an immunomodulatory cytokine such as IL-2 have been reviewed.[52] In terms of leukaemia, transduction of the two cytokines IL-2 and IL-7 into a cell line generated from an acute leukaemia patient has shown that in combination these two cytokines are capable of generating both allogeneic and autologous cytotoxic lymphocytes against the leukaemic clones, but not when each cytokine was expressed singly.[53] Successful anti-leukaemia-specific T-cell responses have also been reported for several different leukaemia cell lines and primary AML blasts using combined gene therapy delivering B7.1 and GM-CSF using either monocistronic or bicistronic lentiviral vectors.[54] Other combined therapies under current investigation for AML are B7.1 and IL-2 using the fusagene technology;[55] and B7.1 and IL-12 using a single cDNA that codes for a monomeric polypeptide Flexi-IL-12 which has been packaged into a rec adeno-associated virus and shown to infect AML blasts successfully.[56]

Until recently, limitations of gene delivery systems using vectors such as retroviruses have largely dictated the clinical application of gene therapy. However more recent development of vectors, such as those derived from lentivirus, in combination with viral concentration strategies can now achieve up to 95% infection efficiency of several different leukaemia samples, including primary AML blasts to express B7.1[54] and our own unpublished data (Lucas Chan, personal communication). Novel strategies for concentrating viruses using paramagnetic particles is now proving to be one of the most promising means of providing high-titre vectors to achieve high transduction efficiency of several leukaemic cell lines.[57] However, notwithstanding the advances in gene therapy, the cost and safety of all gene therapy applications remains of paramount concern restricting its widespread applicability.

DENDRITIC CELLS AND VACCINATION STRATEGIES

A new approach for immunotherapy of cancer exploits the use of professional APC of the immune system, mainly dendritic cells (DC). DC have the unique ability to stimulate naïve T cells to mount antigen-specific immune responses, including specific tumour immunity. Several reviews on the immunobiology and properties of DC are available and the reader is referred to these for further details.[58–61] DC have now moved to the centre stage of active cancer immunotherapy.[62–70] For DC-based cancer vaccines, the DC need to be loaded *ex vivo* with tumour antigens and then injected as cellular vaccines. Several protocols have been described for this purpose[66] including:

(1) Pulsing DC with peptides derived from tumour antigens.
(2) Pulsing DC with whole recombinant tumour antigens.
(3) Pulsing DC with antigens derived from tumour cell lysates.
(4) Pulsing DC with apoptotic tumour cell bodies.
(5) Loading DC with tumour-derived RNA or DNA and
(6) Fusing DC directly to tumour cells.

Pre-clinical studies using several different animal models have demonstrated that DC, when loaded *ex vivo* with tumour antigens by one of the above methods, and administered to tumour-bearing hosts, can elicit T-cell-mediated tumour destruction.[62–70] Moreover, DC-based immunization can lead to immunological memory with protection against subsequent tumour challenges.

Animal models for immunotherapy using DC-based vaccines have led to the design of clinical trials to investigate the immunological and clinical effects of antigen-loaded DC administered as a therapeutic vaccine to patients with cancer. To date, over 30 clinical trials have been submitted in the USA, all evaluating the efficacy of DC-based cancer vaccines in the immunotherapy of several different cancers including prostate, melanoma, non-Hodgkin's, breast, renal cell carcinoma, etc. (for full details browse http://clinicaltrials.gov/).

In line with other work from several investigators, we have been developing a DC-based approach specifically for AML immunotherapy.[71] We compared the efficacy of DC-based vaccines loaded with leukaemia-derived antigens by three different approaches: DC pulsed with apoptotic leukaemia cells, DC pulsed with lysate antigens derived from the leukaemia cells and DC fused directly to the leukaemia cells to generate DC-leukaemia hybrid vaccines. Our study showed that all three approaches induced anti-leukaemia immunity with CTL responses that were both MHC-class I restricted and antigen specific.[72] In addition, the DC-leukaemia hybrid vaccines induced the most potent antileukaemia response. We argue that the hybrid approach is very applicable for immunotherapy of leukaemias. Not only is it easy to obtain large number of blasts from the

patient but in addition there are no requirements for the mechanical or chemical disruption of tissues to obtain single cells: a prerequisite for the generation of the DC-hybrid cells from solid tumours. In addition, the hybrid approach allows the delivery into the DC of all the tumour-associated antigens which potentially can induce a polyvalent immune response. With the hybrid approach there is no need to identify the HLA haplotype of the patient, which is required for other approaches such as the pulsing of DC with specific peptides or antigens. In addition, the hybrid technology is easy, cheap and reliable; and it also avoids health and safety issues involved with techniques that rely on the genetic transfer of material by vectors such as viruses. We have now evaluated the efficacy of DC-leukaemia hybrids and other DC-based vaccines as a therapeutic approach in a murine model using the EL4 thymoma cell line expressing ovalbumin as a model antigen (manuscript in preparation).

Other studies using *ex vivo* antigen-loaded DC specifically for AML immunotherapy have also led to promising results. Fujii *et al.* were amongst the first to demonstrate that $CD34^+$-derived DC clusters pulsed with autologous irradiated AML blasts induced anti-leukaemic CTLs.[73] A similar study is now being undertaken by Chevallier *et al.*[74] In a different study, DC pulsed with the A2-restricted MUC1-derived peptide induced MUC1-specific CTLs capable of lysing AML targets that constitutively expressed A2 and MUC1[19]. DC vaccination studies on other types of leukaemias and lymphomas have also revealed the potency of DC immunotherapy and have included DC pulsed with bcr/abl peptides for CML patients[75] and DC pulsed with PML/RAR peptides in acute promyelocytic leukaemia.[76] In a murine model of leukaemia, DC pulsed with apoptotic leukaemia cells were also shown to protect mice against leukaemia development.[77] Induction of autoimmune diseases by DC immunization must of course be considered with immense precaution in any immunotherapy protocol. Although very little information is available on this matter, it has been a subject of debate since the concept of DC immunotherapy was first discussed. For example a leukaemia murine study by Roskrow *et al.* revealed that intensive stimulation of the immune system by modified DC vaccination not only leads to tumour erosion but also to destruction of cells bearing normal self-antigens.[78] The mice with the most intensive regime (DC/IL-2/CD40L treatment) developed a severe systemic autoimmune disorder that resembled GVHD. Although several clinical trials are ongoing for tumour immunotherapy, many more studies are needed to optimize our current protocols. Differentiating AML into DC, an alternative approach to the aforementioned therapy of AML, takes advantage of the myeloid lineage of the AML cells themselves and their ability to differentiate into leukaemia-derived DC. Several investigators have now shown that under the influence of GM-CSF and IL-4 in combination with tumour necrosis factor-a (TNF-α), and CD40L or FLT3-L, AML cells can differentiate into DC, irrespective of their FAB classification and clinical status.[79–83] Under these conditions, the AML-differentiated DC unlike their original blasts, express molecules typical of DC markers including CD1a, CD83, B7.1 and B7.2, CD40 and high-class I and class II. In these studies, the AML-differentiated DCs have been shown to be effective at stimulating both allogeneic and autologous T-cell proliferative responses. In addition, these leukaemia-derived DC have been able to stimulate the generation of anti-leukaemia cytotoxic cells capable of lysing the autologous AML blasts. These encouraging data have now led to further investigations using up to 11 different cytokine combinations for the optimal cytokine requirement for leukaemia-derived DC in short-term cultures as a practical strategy for immunotherapy of leukaemia.[84]

TARGETING AML BY ANTIBODY THERAPY

Antibody therapy for cancer offers immense potential for specific targeting. In addition, for haematological diseases such as AML, the blood and bone marrow cells are readily accessible to intravenously injected substances. For this purpose antibodies have been used either naked, i.e. unconjugated, or bound to radioisotopes or toxins. Two target antigens that are being exploited for AML immunotherapy are CD33 and CD45.[5]

A novel emerging approach for antibody-mediated therapy for AML is the differentiation of AML blasts and AML cell lines by the use of anti-CD44 monoclonal antibodies.[85,86] Such antibodies can induce terminal differentiation, inhibit proliferation and induce apoptosis of the leukaemic lines. However, a cautionary study suggests that these agents should not be administered prior to apoptosis-inducing drugs.[87]

CONCLUSION AND FURTHER REMARKS

There is no doubt that a new era has emerged for cancer immunotherapy. A breath of fresh air and new optimism have been waived in with the recognition of what constitutes antigen-specific immunity, the discovery of new antigens, state-of-the-art technology for immune monitoring, and clinical responses in selected patients undergoing clinical trials. The current approaches described in this review are aimed at achieving successful immunotherapy in AML despite any immunosuppression associated with this disease.[88,89] Complimentary reviews on AML immunotherapy have been listed previously.[5–7,90]

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