The immune system as a foundation for immunologic therapy and hematologic malignancies: a historical perspective

Frédéric Baron
Research assistant, National Fund for Scientific Research, Belgium
Fred Hutchinson Cancer Research Center, and the University of Liège, Belgium

Rainer Storb* MD
Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, D1-100, PO Box 19024, Seattle, WA 98109-1024, USA, and the University of Washington, Seattle, USA

In this review we aim to provide a historical overview of the immunotherapeutic approaches which have been developed for the treatment of hematological malignancies. After briefly summarizing the development of the theory of cancer immune surveillance, we describe how initial studies discovering the efficacy of the immune-mediated graft-versus-tumor effects after allogeneic hematopoietic cell transplantation led to new transplantation approaches (termed non-myeloablative transplantation) relying almost exclusively on graft-versus-tumor effects for tumor eradication. We then summarize important steps in the development of tumor vaccines and autologous adoptive immunotherapy in patients with hematological malignancies. Finally, we describe historical discoveries leading to the recent success with monoclonal antibodies as treatment for lymphomas, chronic lymphocytic leukemia, and acute myeloid leukemia.

Key words: hematopoietic cell transplantation; immunotherapy; graft-versus-tumor effects; monoclonal antibody.

The notion of immunologic surveillance was first hypothesized in 1909 by Paul Ehrlich who proposed that during fetal and post-fetal development aberrant cells occurred frequently, but were eliminated or remained latent due to control by the immune system.1,2 Fifty years later, Thomas and Burnet developed the ‘immunologic surveillance’ theory which proposed that, under normal circumstances, the immune system
destroyed cancer cells when they were still in the early stages of tumor formation, probably because tumor cells differed antigenically from normal cells. Among the arguments in favor of this theory at that time were (1) the increased incidence of both hematologic and solid tumors observed in thymectomized mice and patients with primary immunodeficiencies; (2) the increased incidence of neoplasia in patients given immunosuppressive therapy (for example after organ transplantation); and (3) the demonstration that mice could be immunized against syngeneic tumors induced by viruses or chemical carcinogens.

Studies in recently characterized athymic nude mice in the 1970s seriously challenged the cancer immunosurveillance theory. Indeed, although it was evident that thymus-deficient mice had increased incidences of lymphoma and virally induced tumors, they were not more susceptible to spontaneous or chemically induced tumors. These observations led to a progressive loss of interest in the immunosurveillance theory. However, the theory was resurrected in the 1990s because of four key observations. First, interferon-γ (IFN-γ) was shown to prevent both chemically induced and spontaneous tumors. Second, mice lacking the perforin (perforin −/−) gene, and mice deficient for the recombination-activating gene 1 (RAG-1) or RAG-2 (leading to a complete lack of T cells and B cells without affecting non-lymphoid organs) were shown to develop chemically induced tumors more frequently than their wild-type counterparts. Third, further studies in mice demonstrated that both the innate (NK-cell) and adaptive (T-cell) immune systems could be implicated in immune surveillance, since mice deficient for NK cells, T cells, NK/T cells, IFN-γ or interleukin 12 (IL12) all had increased susceptibilities to tumors (reviewed by Dunn et al). Finally, it was proposed that the immune system not only protected hosts again tumor development, but also facilitated outgrowth of tumors with reduced immunogenicity (the tumor editing hypothesis, recently reviewed by Dunn et al).

Although documented in mice, the importance of cancer immune surveillance in humans for prevention of non-viral tumors has remained highly controversial. Nevertheless, a significant role for cancer immune surveillance in humans has been suggested by epidemiological data showing increased incidences of both lymphoma and various carcinomas in solid-organ graft recipients given immunosuppressive therapy and in patients with acquired immune deficiency syndromes.

In parallel with — and with no initial relation to — the immune surveillance theory, allogeneic hematopoietic cell transplantation (HCT) was introduced by Thomas et al and Mathé et al as potential treatment for patients with hematologic malignancies. While the aim of the procedure was initially to administer supra-lethal doses of irradiation with the hope of destroying all leukemic or abnormal cells, it was recognized in the late 1970s that allogeneic immunocompetent cells transplanted with the stem cells mediated therapeutic anti-tumor effects which were independent of the action of the irradiation, termed graft-versus-tumor effects. These observations encouraged the study of new strategies of immune therapy for hematological malignancies via infusions of T cells specific for tumor antigens, or via vaccination against leukemia-associated antigens (see the excellent review by Morris et al).

In addition to the progress made with cellular-based immunotherapy, advances in the field of tumor-targeting monoclonal antibodies (mAbs) have taken place in recent years, as illustrated by the development of chimeric mAbs targeting the CD20 antigen, which are now used in all types of B-cell non-Hodgkin lymphomas (NHLs), or CD52 or CD33 antigens, used increasingly for the treatment of chronic lymphocytic leukemia (CLL) or acute myeloid leukemia (AML) respectively. In addition, β-emitting radio nuclides conjugated to mAb directed against the CD45 antigen (expressed on all
hematopoietic cells) have been investigated as a way of increasing the anti-leukemic potency of conditioning regimens for HCT without inducing undue systemic toxicities.\textsuperscript{25,26}

The aim of this chapter is to provide a brief historical overview of immunotherapeutic approaches developed to treat hematological malignancies. We have divided the chapter into three sections: (1) immunotherapy with allogeneic HCT; (2) autologous cell-based immunotherapy and vaccines; and (3) antibody-based therapies. However, these strategies are not independent from each other, as illustrated by recent studies combining mAbs with allogeneic HCT.\textsuperscript{25–27}

**IMMUNOTHERAPY WITH ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION**

**Demonstration of graft-versus-tumor effects in rodents and humans**

Several studies have demonstrated graft-versus-tumor effects in rodent models. In 1957 Barnes and Loutit first showed that mice with leukemia could not be cured by total body irradiation (TBI, 9.5 Gy) and infusion of syngeneic marrow, whereas mice given allogeneic marrow after the same dose of TBI survived for longer periods of time without evidence of leukemia, although eventually almost uniformly dying of graft-versus-host disease (GVHD).\textsuperscript{28} They proposed that a reaction of the donor marrow killed leukemic cells. This reaction was termed graft-versus-leukemia effect by G. Mathe in 1965.\textsuperscript{17} Further studies showed that, while transplantation of H-2-incompatible marrows caused GVHD and prevented leukemia relapses, transplantation of immunocompetent cells from syngeneic or from H-2-identical murine donors did not cause GVHD, but did not produce detectable graft-versus-tumor effects either.\textsuperscript{29}

The first evidence for graft-versus-tumor effects in humans came from a study published in 1979 showing a 2.5-fold lower risk of leukemic relapse rates in allogeneic marrow recipients who developed acute GVHD in comparison with those who did not.\textsuperscript{18} However, this did not translate into improved progression-free survival, since acute GVHD was associated with increased non-relapse mortality. Two years later, the same authors observed that chronic GVHD, a disease mimicking autoimmune disorders and generally occurring late after HCT, was also associated with a reduced risk of leukemic relapse and improved progression-free survival in patients with advanced acute leukemia given allogeneic marrow grafts.\textsuperscript{19} Interestingly, the highest survival rate was observed in patients with chronic GVHD who did not have acute GVHD (de novo chronic GVHD, Figure 1). The anti-leukemic effects of acute and chronic GVHD were confirmed in 1990 in a large study from the International Bone Marrow Transplant Registry (IBMTR).\textsuperscript{30} Further, patients given syngeneic marrow were shown to have increased risks of relapse in comparison to patients given allogeneic marrow who did not develop GVHD, demonstrating that graft-versus-tumor effects could be dissociated from clinically apparent GVHD.\textsuperscript{18,30} Finally, the important role of donor T cells in the graft-versus-tumor effect was highlighted by reports demonstrating that, although the incidence of GVHD was decreased following transplantation of T-cell-depleted marrow, graft failure and leukemia relapse were significantly increased by this procedure.\textsuperscript{30–32}

First attempts at increasing graft-versus-tumor effects in patients with high-risk leukemia by shortening the duration of post-grafting immunosuppression or by infusing donor buffy coats early after transplantation were not successful because of increased incidence of severe acute GVHD and increased non-relapse mortality.\textsuperscript{33}
Donor lymphocytes infusion (DLI)

Given the primary role of donor T cells in the graft-versus-tumor effect and the observation that patients who relapsed after transplantation occasionally achieved complete remission during flares of GVHD activity, Kolb et al. investigated the efficacy of donor lymphocyte infusions (DLI) in patients with leukemia relapse after allogeneic transplantation. The induction of durable complete remissions by DLI in a number of patients with either acute or chronic leukemia, multiple myeloma, or lymphoma demonstrated that graft-versus-tumor effects were capable of eradicating hematological malignancies, even in the absence of preceding chemotherapy.

Two large multicenter studies have analyzed outcomes after DLI in more than 400 patients with relapse after HCT. DLI induced sustained complete remissions in more than 60% of patients with chronic myeloid leukemia, and in 10–40% of patients with other hematologic malignancies. Acute GVHD and chronic GVHD were each associated with increased probabilities of disease responses, although some patients achieved complete remissions without clinically evident GVHD.

Non-myeloablative and reduced-intensity conditioning

Due to regimen-related toxicities, the use of conventional (myeloablative) allogeneic HCT has been restricted to younger and medically fit patients. This is
Unfortunately since the median ages at diagnosis of patients with most hematological malignancies ranges from 65 to 70 years (Table 1). Given the increasingly recognized importance of graft-versus-tumor effects for controlling cancer, several groups of investigators have explored allogeneic HCT after reduced-intensity or truly non-myeloablative conditioning regimens \(^{44-49}\) in which some or all the burden of tumor eradication was shifted from high-dose chemoradiotherapy towards graft-versus-tumor effects. \(^{50}\)

On the basis of preclinical observations in a canine model of transplantation \(^{51}\), we have been investigating a very-low-intensity regimen combining low-dose TBI (2 Gy) with or without added fludarabine (90 mg/m\(^2\)) and post-grafting immunosuppression with mycophenolate mofetil and cyclosporine. \(^{46,52,53}\) To date, more than 800 patients have been given allogeneic HCT after this non-myeloablative conditioning regimen on clinical trials carried out in several centers in the US and Europe, as described in the following paragraphs.

The regimen has been usually very well tolerated, and has been associated with fewer infections \(^{54}\) and less transplant-related toxicity \(^{55-61}\) than myeloablative conditioning, despite the fact that non-myeloablative conditioning was offered in older patients and those with medical comorbidities. Characteristics of the regimen included modest declines in peripheral-blood cell counts \(^{46,55}\), and gradual replacement of recipient hematopoiesis by donor-derived hematopoiesis. \(^{62,63}\) Acute GVHD of grades II, III and IV was seen in 33%, 10% and 5% of patients given grafts from HLA-matched related donors (MRDs), compared with 41%, 9% and 3% in those given grafts from unrelated donors (URDs), respectively. \(^{64}\) Chronic GVHD occurred in 43% of MRD and in 45% of URD recipients. \(^{64}\) In comparison with patients given myeloablative conditioning, grade II–IV acute GVHD was significantly less frequent in non-myeloablative recipients, but the incidences of chronic GVHD were similar among patients given non-myeloablative or myeloablative conditioning. \(^{65}\)

### Table 1. Median ages of patients at diagnoses and at hematopoietic cell transplantation (HCT) using myeloablative or non-myeloablative conditioning.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Median ages of patients (years)</th>
<th>At diagnoses (SEERS) (^{41})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Related donors</td>
<td>Unrelated donors</td>
</tr>
<tr>
<td></td>
<td>Myeloablative conditioning(^a)</td>
<td>Non-myeloablative conditioning(^b)</td>
</tr>
<tr>
<td>CML</td>
<td>40 (^{41}) 58 (^{70})</td>
<td>36 (^{41}) 54 (^{71})</td>
</tr>
<tr>
<td>AML</td>
<td>28 (^{41}) 58 (^{73})</td>
<td>33 (^{41}) 57 (^{73})</td>
</tr>
<tr>
<td>NHL</td>
<td>33 (^{41}) 53.5 (^{68})</td>
<td>35 (^{41}) 53.5 (^{68})</td>
</tr>
<tr>
<td>MM</td>
<td>45 (^{41}) 52 (^{67})</td>
<td>45 (^{41}) 52 (^{106})</td>
</tr>
<tr>
<td>CLL</td>
<td>51 (^{41}) 55 (^{69})</td>
<td>46 (^{41}) 58 (^{69})</td>
</tr>
<tr>
<td>HD</td>
<td>29 (^{41}) 37 (^{107})</td>
<td>28 (^{41}) 37 (^{107})</td>
</tr>
<tr>
<td>MDS</td>
<td>40 (^{41}) 62 (^{73})</td>
<td>41 (^{41}) 62 (^{73})</td>
</tr>
<tr>
<td>Overall</td>
<td>40 (^{41}) 55 (^{64})</td>
<td>35 (^{41}) 55 (^{54})</td>
</tr>
</tbody>
</table>

SEERS, surveillance, epidemiology and end results; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; CLL, chronic lymphocytic leukemia; HD, Hodgkin disease; MDS, myelodysplastic syndrome.

\(^a\) At the Fred Hutchinson Cancer Research Center (FHCRC).

\(^b\) FHCRC consortium.
Sustained tumor responses have been seen across all disease categories. Overall complete responses in patients with measurable disease at HCT approached 50%, while the remainder of the patients had partial responses or stable disease, or experienced disease progression or relapse. Chronic GVHD was associated with decreased relapse/progression ($P = 0.006$), and better progression-free survival ($P = 0.003$). However, grades II and III/IV acute GVHD had no statistically significant impact on relapse/progression. Instead, acute GVHD was associated with increased non-relapse mortality. In agreement with what was observed after myeloablative conditioning, the best progression-free survival was observed in patients with de novo chronic GVHD.

We have recently reported on the efficacy of allogeneic HCT following non-myeloablative conditioning in specific disease categories. Hegenbart et al summarized results of non-myeloablative HCT in 122 patients with acute myeloid leukemia, 117 of whom were ineligible for conventional HCT because of age and/or comorbidities. Median age at HCT was 58 (range 17–74) years. Two-year probabilities of overall survival were 51% for patients transplanted in first complete remission ($n = 51$), 61% for those transplanted in second remission ($n = 39$), and 28% for those transplanted beyond second remission ($n = 32$). Kerbauy et al reported outcomes in 24 patients (median age 58 years) with chronic myeloid leukemia in the first chronic phase ($n = 14$) or beyond the first chronic phase ($n = 10$) given grafts from HLA-matched related donors. Most patients were deemed ineligible for conventional HCT either because of age $\geq 65$ years ($n = 5$), or because of comorbidities ($n = 15$). The 2-year overall survival was 70% for patients transplanted in the first chronic phase and 56% for those with more advanced diseases. Most patients with sustained engraftment achieved molecular remissions (Figure 2). Scott et al compared efficacy of HCT after non-myeloablative ($n = 38$) versus myeloablative conditioning (busulfan and cyclophosphamide, $n = 112$) in patients over 40 years of age with myelodysplastic syndromes. In multivariate analyses, there were no significant differences in overall survival (HR 1.2, $P = 0.56$), progression-free survival (HR 1.1, $P = 0.60$), and relapse risk (HR 1.3, $P = 0.43$) between the non-myeloablative versus myeloablative recipients, suggesting that graft-versus-tumor effects were more important than conditioning intensity in...
preventing relapse in patients with myelodysplastic syndromes. Results in 33 patients with mantle-cell lymphoma given grafts from related (n = 16) or unrelated (n = 17) donors were reported by Maris et al.\textsuperscript{68} Fourteen patients had failed high-dose autologous HCT. Of patients with measurable disease at HCT (n = 20), 85\% achieved partial (10\%) or complete (75\%) remissions (Figure 3). Two-year incidences of relapse, non-relapse mortality, overall survival and progression-free survival were 9\%, 24\%, 65\%, and 60\%, respectively. Finally, Sorror et al reported results of non-myeloablative allogeneic HCT in 64 patients with fludarabine-refractory chronic lymphocytic leukemia\textsuperscript{69}; 44 patients received grafts from related donors, and 20 received grafts from unrelated donors. Of patients with measurable disease at HCT (n = 61), 67\% achieved partial (17\%) or complete (50\%) remissions. Two-year incidences of relapse, non-relapse mortality, overall survival and progression-free survival were 9\%, 24\%, 65\%, and 60\%, respectively.

![Figure 3](example.png)

**Figure 3.** Example of graft-versus-tumor response in a patient with mantle-cell lymphoma treated with allogeneic hematopoietic cell transplantation (HCT) after non-myeloablative conditioning with fludarabine and low-dose (2 Gy) total body irradiation (TBI). At the time of allogeneic HCT the patient had recurrent disease following treatment with high-dose radiolabeled antibodies and autologous peripheral-blood stem-cell support. (A) Pretransplantation computed tomography (CT) scan image (day −27) through the upper pelvis demonstrating a mass 8 cm × 7 cm extending through 12 0.5-cm cuts. (B) CT scan image through the same region demonstrating complete resolution of the mass on day +74 after non-myeloablative transplantation from an HLA-matched unrelated donor. The patient has remained in remission 30 months after transplantation with no evidence of graft-versus-host disease (GVHD). From Maris et al (2004, Blood 104: 3535–3542) with permission.
mortality, overall survival and progression-free survival were 18%, 22%, 60%, and 52%, respectively.

**NK cell alloreactivity: lessons from HLA-mismatched HCT**

In the late 1970s a subpopulation of lymphocytes — termed natural killer (NK) cells — was described that could spontaneously kill (i.e. without previous immunization) a variety of target cell types, usually tumor cells or allogeneic hematopoietic cell lines. Those cells were believed to play a role in immunosurveillance against leukemias, genetic resistance to marrow allografts, and resistance to viral infections. However, although NK activity was found to be low in patients with leukemias, possibly as a result of the presence of blasts diluting or replacing NK cells, it was observed that NK function returned to normal by day 30 after allogeneic HCT, and that NK function did not correlate with subsequent risk of relapse, suggesting that NK cells did not play a significant immunosurveillance role against leukemia after HLA-identical sibling HCT. Moreover, analyzing data from patients with aplastic anemia, there was no correlation between host NK-cell activity before HCT and the risk of graft rejection, suggesting that NK cells did not play a significant role in the graft rejection process after HLA-identical sibling HCT. Subsequent observations in patients with severe combined immunodeficiency lacking T-cell function but having NK-cell function confirmed earlier canine studies showing that NK cells were not involved in graft rejection of HLA-identical sibling marrows but played an important role in rejecting unrelated or MHC-haploidentical grafts.

The identification of mechanisms regulating NK-cell activity in the mid 1990s renewed interest in NK cells. The activity of NK cells was regulated by a quantitative balance between inhibitory signals mediated by inhibitory killer immunoglobulin-like receptors (KIRs) and CD94/NKG2A, and by activating signals mediated by natural cytotoxicity receptors (NCRs), including recently identified NKG2D or DNAX accessory molecule-1 (DNAM-1, CD226). The mechanisms of NK-cell inhibition were also identified. KIRs recognized allotypic determinants shared by different HLA-class I alleles: KIR2/DL2 and KIR2/DL3 recognized HLA-C group 1 alleles, KIR2/DL1 recognized HLA-C group 2, and KIR3/DL1 recognized HLA-Bw4 alleles. Conversely, CD94/NKG2A recognized overall expression of HLA class I molecules on target cells via the expression of HLA-E molecules on their surface. It was postulated that most tumor cells that lacked HLA class I molecules were promptly killed by NK cells because of the predominant effect of several activating receptors such as NKG2D or DNAX. It was also shown that the ligands for NK activating receptors were over-expressed on the surface of many tumor cells.

These discoveries prompted Velardi et al to study the role of NK cells after HLA-haploidentical (donor and recipient pairs identical for one HLA haplotype but fully mismatched for the unshared haplotype) HCT. The authors proposed that all mature NK cells expressed at least one inhibitory receptor for self-HLA, and thus that the presence or absence of functioning KIRs could be deduced by HLA-genotype. They then presented a simple algorithm in which comparisons between the donors' and recipients' HLA genotyping allowed prediction of NK alloreactivity (KIR ligand incompatibility model). Applying this model in patients given HLA-haploidentical HCT, they found that donor-versus-recipient NK-cell alloreactivity was associated with reduced risks of graft rejection, lower incidences of acute GVHD, and lower risks of relapse in patients with acute myeloid leukemia but not in acute lymphoblastic
Several groups of investigators subsequently tested the KIR ligand incompatibility model in patients given grafts from HLA-mismatched unrelated donors. Two studies found lower risks of relapse in patients with KIR ligand incompatibility in the graft-versus-host direction, while two others did not find such associations.

Given that HLA and KIR genes are on chromosomes 6 and 19, respectively, they are inherited independently. In addition, phenotyping of peripheral NK cells has demonstrated that the HLA genotype has only a subtle impact on KIR expression on peripheral NK cells. These observations were the basis for the 'missing KIR ligand model' in which donor—recipient NK-cell alloreactivity was predicted by analysis of donor KIR genotype and recipient HLA genotype. Because HLA and KIR segregate separately, the missing KIR ligand model could be applicable to HLA-identical HCT. Suggesting a potential role for NK cells in patients with acute myeloid leukemia given grafts from HLA-identical recipients, Hsu et al found a lower risk of relapse and better disease-free survival among patients who lacked the HLA ligand for one or more donor inhibitory KIR, in comparison to patients who had all of the HLA ligands.

AUTOLOGOUS CELLULAR-BASED IMMUNOTHERAPY AND VACCINES

The concept of using autologous adoptive immunotherapy or cancer vaccine has had a long history of success in rodent models, but until now these successes have not been fully translated in humans. However, the discoveries of potential tumor-associated antigens and advances in tumor immunology have opened the way for the development of more effective strategies.

Tumor-associated antigens and transfer of tumor-specific T cells

The existence of tumor-specific antigens was first demonstrated in the 1950s by studies showing that mice could be immunized against syngeneic tumors induced by chemical carcinogens or oncoviruses. While the lower incidences of relapse after allogeneic HCT compared with syngeneic HCT demonstrated the importance of T-cell responses to minor histocompatibility antigens as targets of graft-versus-tumor effects, other types of protein exclusively expressed by tumor cells — such as the immunoglobulin idiotype (Ig-Id) in B-cell lymphoma or multiple myeloma, bcr/abl protein in chronic myeloid leukemia (CML), or Epstein—Barr-virus- (EBV-) associated proteins in Hodgkin disease or Burkitt's lymphoma — or protein over-expressed by tumor cells (such as proteinase-3, or WT-1), were thought to also play roles in graft-versus-tumor effects. For example, Molldrem et al observed a strong correlation between T-cell responses directed against proteinase 3 and clinical responses of chronic myeloid leukemia cells to interferon-γ or allogeneic HCT.

By optimizing culture conditions it became possible to isolate from normal donors or from leukemia patients infrequent T cells which had cytotoxic activity against such tumor-associated antigens both in vitro and in xenotransplantation models. Studies in the early 1990s showed that it was possible to restore T-cell immunity to cytomegalovirus (CMV) after allogeneic HCT by transfer of CMV-specific T cells. Similar results were obtained with EBV as target. However, generating sufficient numbers of tumor-specific T cells with a high affinity against patient-specific tumor-associated
antigen, and assuring their persistence after injection, have remained challenging. These limitations might be overcome by recent approaches that have used genetic modifications of T cells, for example by transfer of genes coding for tumor-specific T-cell receptors with or without genes coding for signaling domains of co-stimulatory molecules (see excellent reviews by Morris et al and Rossig and Brenner).

Vaccination

A recent review of vaccination trials performed in over 400 patients with solid cancer (mainly metastatic melanomas) showed an objective response rate of only 2.6%. However, more encouraging results have been observed in patients with non-Hodgkin B-cell lymphoma (NHL) by targeting the tumor-specific Ig-Id, with 20–35% of patients achieving objective responses.

B-cell NHL has been an ideal target for tumor vaccines because malignant B cells express both co-stimulatory molecules and a highly immunogenic target antigen (the Ig-Id). Early observations in 1982 provided proof of principle that the immune system was able to target Ig-Id and kill Ig-Id-positive (lymphoma) cells, since mAbs directed against the Ig-Id induced tumor regression and even sustained clinical remissions in a subset of patients with NHL. However, in a number of patients tumors recurred which originated from cells that contained mutations of the unique Ig-Id antigen that was recognized by the mAbs. This prompted investigations of specific Ig-Id vaccination in the early 1990s. Researchers at Stanford University pioneered this strategy, and used Ig-Id conjugated to the carrier protein keyhole limpet hemocyanin (KLH), and an adjuvant (Syntax adjuvant formulation). This strategy led to polyclonal antibody and T-cell responses directed against several Ig-Id epitopes and resulted in a decreased risk of tumor escape. Other investigators added injection of granulocyte-macrophage-colony-stimulating factor (GM-CSF) in order to promote immune responses. Results of phase-II studies in patients with follicular NHL who were in complete remission at the beginning of the trial suggested effectiveness of the vaccine in promoting anti-Ig-Id immunity and clinical efficacy. This has led to the development of phase-III clinical trials. Similarly, Ig-Id immunization has led to anti-idiotype immunity in patients with multiple myeloma, although apparently this has as yet not translated into improved survival.

One strategy to increase antigen presentation has consisted of first pulsing maturing dendritic cells in vitro and then administering the cells, followed by Ig-Id protein boosts. This has resulted in objective responses in 20–35% of patients with B-cell NHL who had measurable disease at the time of the vaccination.

Ongoing studies are testing efficacy of vaccines directed against other tumor-associated antigens, such as BCR/ABL protein or proteinase 3. Another approach involved injecting autologous or allogeneic tumor cells that were genetically modified to secrete cytokines (such as GM-CSF) locally. This might allow generation of immunity against several tumor-selective antigens (polyvalent vaccination), thereby reducing the risk of antigen escape of tumor cells.

MONOClonAL ANTIBOdIES

The development of murine and rat hybridoma technologies in the 1970s has allowed the production of mAbs of predefined specificity. Despite the relative success with anti-mAbs directed against Ig-Id (see above), most rodent mAbs directed against...
human myeloid, B-cell or T-cell antigens have failed to produce significant responses in vivo.104 These studies also revealed the deleterious effects of human anti-mouse antibodies (HAMA).104 In 1994, it was shown that adding the human IgG1 Fc region to murine mAb allowed more efficient complement-dependent cytotoxicity (CDC) and increased antibody-dependent cellular cytotoxicity (ADCC) in comparison with the murine Fc region.105 The use of human chimeric mAb has decreased host anti-mAb responses, and increased the mAbs’ serum half-lives.105

Another important consideration for the efficacy of mAbs has been the characteristics of the target antigens. Ideal antigens should be expressed at high density on the tumor cells (>10000 binding sites per cell) and be weakly expressed by normal cells.104

Two different groups of humanized mAbs have been developed. The first group of mAbs has targeted antigens (such as CD20, CD45 or CD52) that were slowly or minimally internalized after binding and relied on extracellular mechanisms of cytotoxicity such as CDC or ADCC, or on being conjugated with β-emitting radionuclides. Examples of humanized antibodies directed against such targets currently in clinical use include rituximab (anti-CD20)21 and alemtuzumab (anti-CD52).22 An example of a radiolabeled mAb is a 131I-anti-CD45 antibody used before allogeneic HCT.25

The second group of mAbs was directed at target antigens that are internalized after binding, such as CD33. Internalization has been required for those mAbs that were developed for delivering toxins or chemotherapeutic drugs into the cytoplasm of tumor cells. An example of a humanized mAb in clinical use is gemtuzumab ozogamicin, a mAb that targets CD33 and is conjugated with calicheamicin, a highly potent antitumor antibiotic that cleaves double-stranded DNA at specific sequences.23

SUMMARY

During the past 50 years, the use of the immune system to destroy hematological malignancies has moved from a hypothesis to an effective therapy for thousands of patients. Efficacy of immunotherapy has been demonstrated in patients with hematological malignancies undergoing allogeneic HCT following non-myeloablative conditioning. Independent from advances in allogeneic HCT, MAbs targeting CD20, CD52, CD45 or CD33 have been developed and become important therapeutic tools for B-cell NHL, chronic lymphocytic leukemia, and acute myeloid leukemia. Finally, recent progress in tumor immunology might lead to successful tumor vaccines and to the development of highly cytotoxic autologous tumor-specific T cells.

Practice points

- allogeneic immunocompetent cells transplanted with the graft mediate therapeutic anti-tumor effects after allogeneic HCT (graft-versus-tumor effect)
- efficacy of graft-versus-tumor effect has been demonstrated in patients with hematological malignancies undergoing allogeneic HCT following non-myeloablative conditioning
- monoclonal antibodies targeting CD20, CD52, CD45 or CD33 have been developed and become important therapeutic tools for the treatment of B-cell non-Hodgkin lymphoma, chronic lymphocytic leukemia, and acute myeloid leukemia
ACKNOWLEDGEMENTS

This work was supported by grants CA78902, CA18029, CA15704, DK42716, and HL36444 of the National Institutes of Health, Bethesda, MD, USA. Frédéric Baron is research assistant of the National Fund for Scientific Research (FNRS) Belgium and supported in part by postdoctoral grants from the Fulbright Commission.

REFERENCES


52. Niederwieser D, Maris M, Shizuru JA et al. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematologic diseases. Blood 2003; 101(4): 1620–1629.


77. Raff RF, Sandmaier BM, Graham T et al. ‘Resistance’ to DLA-nonidentical canine unrelated marrow grafts is unrestricted by the major histocompatibility complex. *Experimental Hematology* 1994; **22**: 893–897.


88. Frohn C, Schlenke P & Kirchner H. The repertoire of HLA-Cw-specific NK cell receptors CD158 a/b (EB6 and GL183) in individuals with different HLA phenotypes. *Immunology* 1997; 92(4): 567–570.


91. Bishara A, De Santis D, Witt CC et al. The beneficial role of inhibitory KIR genes of HLA class I NK epitopes in haploidentically mismatched stem cell allografts may be masked by residual donor-alloreactive T cells causing GVHD. *Tissue Antigens* 2004; 63(3): 204–211.


