Bone Marrow Transplantation (2002) 30, 549–556

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Meeting report

New concepts about graft-versus-host and graft-versus-leukaemia-reactions. A summary of the 5th International Symposium held in Munich, 21 and 22 March 2002

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Summary:

The Fifth International Symposium on Graft-versus-Host and Graft-versus-Leukemia Reactions was held on 21 and 22 March 2002 in the University Hospital (Klinikum Grosshadern) of the University of Munich. As in previous years, it was dedicated to the encounter of scientists and clinicians involved in hematopoietic cell transplantation. This year's symposium focused on the characterization of stem cells potentially expanding the use of hematopoietic stem cells and on gene therapy. The immunology section dealt with mechanisms of tolerance, and the characterization of minor histocompatibility antigens presented by major histocompatibility molecules. Further important topics were cytokines and dendritic cells. In 1½ days of intense work, the invited speakers, chairmen, authors and an active audience experienced an exciting exchange of ideas and collaboration. Again, new impulses were given for basic research and clinical transplantation. The authors would like to express their deep appreciation and thanks to all participants of this symposium.
Bone Marrow Transplantation (2002) **30,** 549–556. doi:10.1038/sj.bmt.1703690
Keywords: graft-versus-host-reactions, graft-versus-leukaemia-reactions, gene therapy, immunotherapy

As in previous years, the major topics were covered by invited lectures from out-standing experts. For the first time a poster session was included and provided the participants with the opportunity to present data. The authors of the best posters were invited to participate in a panel discussion at the end of each session. The program was structured to leave sufficient time for questions and the panel discussions. On the last day, a panel of experts summarized the most interesting findings and gave directions to future research. Taken together, the productive atmosphere of a workshop was maintained throughout the symposium.

In this edited review, we summarize the major concepts and data (17 invited lectures and eight selected posters) presented at the 2002 symposium.

Malcolm Brenner (Baylor College of Medicine, Houston, TX, USA) gave the introductory lecture. He presented an overview (from bone marrow transplantation to cell and gene therapy) and showed new data on gene transfer in hematopoietic stem cell transplantation. Basically, gene transfer may optimize cellular therapies targeting infectious agents or malignancies, improve the performance of stem cell transplantation and enhance tolerance of recipient antigens by donor cells without ablating the antileukemic response.

Specific cellular therapies have been applied in cases of EBV-associated malignancies. There are three types of EBV latencies: type 1 is associated with Burkitt's lymphoma, type 2 is associated with Hodgkin's disease and nasopharyngeal carcinoma, and type 3 is associated with post-transplant lymphoproliferative disease. Post-transplant lymphomas arise in the context of immunosuppression and T cell-depleted allografts. The group from Houston prepared genetically marked cytotoxic T lymphocytes (CTL) specific for EBV (for details see Figure 1). As stimulator cells, EBV-transformed lymphoblastoid cell lines were prepared from donor B lymphocytes. As a pre-emptive strategy, CTL were infused into patients in whom the viral load of EBV increased in the peripheral blood. Until now, none of the more than 100 patients who have been treated have developed post-transplant lymphoma, whereas in historical controls an incidence of about 12% was found. CTL were also shown to be effective in established post-transplant lymphomas. These studies serve as a paradigm for T cell immunotherapy of other malignancies. In type 2 disorders like Hodgkin's disease, immunotherapy is more difficult, since the malignant cells express fewer EBV antigens. At total of eight patients with relapsed Hodgkin's disease and four patients at risk of relapse have been treated until now with CTL. Mixed responses were observed in seven patients and a partial remission in one. Present work aims at making CTL more specific and at understanding the

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Received 28 May 2002; accepted 28 June 2002

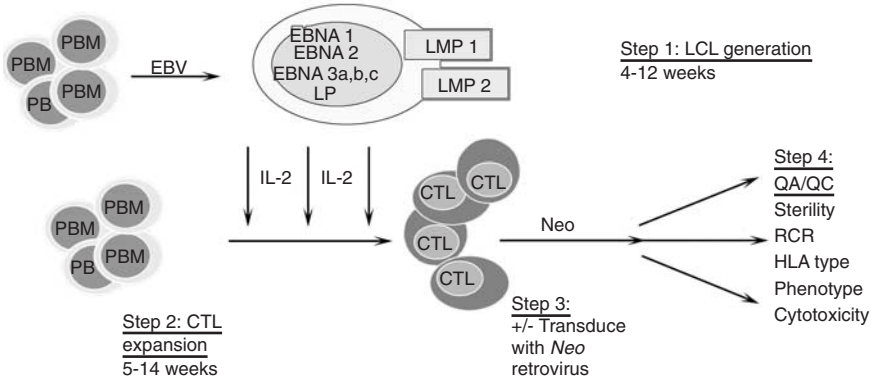

Figure 1 Generation of genetically marked EBV-specific cytotoxic T lymphocytes for the treatment and prophylaxis of post-transplant lymphomas.

immune evasion strategies employed by malignant cells. A further approach is to use chimeric T cell receptors. Engineered CTL expressing a chimeric receptor directed against a tumor-associated antigen proliferate long-term in the presence of EBV lymphoblastoid cell lines and respond by cytokine release and target cell killing when exposed to either EBV or the chimeric-receptor target.[1]

A further application of gene therapy is to modify the capacities of stem cells and stem cell subpopulations. An interesting population of stem cells is the so-called side-population (SP) which is found in all mammalian species and comprises the bulk of murine marrow repopulating cells. These cells may also mediate transdifferentation in human hematopoiesis (eg giving rise to muscle cells, vascular cells and osteoblasts). Based on these considerations, allogeneic transplantation was performed in children with severe osteogenesis imperfecta, a genetic disorder with a defect in type I collagen.[2] It was shown that the mesenchymal cells were actually of donor origin, that the bone mineral content increased and that fewer fractures occurred after transplant. This work was extended by isolating, expanding and infusing mesenchymal cells post transplant. Since these transplants lead to a low level (1–3%) engraftment of mesenchymal cells, future work should focus on making transdifferentiation more efficient. For some genes, a transient expression in stem cells might be helpful. Finally it was shown to be possible to produce regulatory T cells that could specifically inhibit allogeneic responses by expressing Notch ligand genes in B lymphocytes or other antigen presenting cells and thereby induce highly specific donor tolerance.

Fabio Ciceri (Milan, Italy) presented current studies on suicide gene transfer after stem cell transplantation. Donor lymphocytes were engineered with the HSV-TK (herpes simplex thymidine kinase) suicide gene to confer sensitivity to ganciclovir. Retroviral vectors also carrying a selectable surface marker (neomycin resistance and/or low affinity nerve-growth factor receptor) were used for gene transfer. Two groups of patients are being treated: those in relapse after a matched allogeneic transplant and haploidentical T cell-depleted transplants (add-back of the donor cells 6 weeks after transplant). In cases of severe GVHD, giving ganciclovir can then eliminate the marked cells. In the earlier series of experiments, a fraction of patients developed immunization against HSV-TK-transduced cells. In the relapse treatment protocol, HSV-TK-transduced cells engrafted in most patients and remained in circulation for up to 60 months. The transduced cells still had GVL activity. Ganciclovir was effective in eliminating transduced cells and controlling GVHD. So far eight patients who had haploidentical transplants for high-risk disease have been studied. The transduced cells engrafted in seven of eight cases, and with a short follow-up, five of eight maintained a complete remission. One case of acute GVHD grade III after haploidentical transplantation was successfully controlled by ganciclovir alone.

Boris Fehse (Hamburg, Germany) reported insertional mutagenesis occurring in mouse gene marking experiments. No lesion occurred in primary recipients. Secondary recipients of stem cells marked with a retroviral vector encoding a truncated version of nerve growth factor developed a myelodysplastic state which progressed to acute myeloid leukemia. The malignant cells expressed Evi-1 (ecotropic viral integration site-1, a transcription factor involved in human myelodysplasia and myeloid leukemia) and proliferated in response to nerve growth factor. In fact, the myeloid blasts expressed trka, the high-affinity receptor for nerve-growth factor. This observation which was recently published,[3] argues for caution in the genetic engineering of long-lived stem cells. Well-designed animal models and multicenter efforts will be required for systematic risk assessment of side-effects related to transgene integration and expression.

Willem Fibbe (Leiden, The Netherlands) discussed the role of mesenchymal stem cells (MSC) in promoting the engraftment of hematopoietic stem cells. Earlier studies had shown a high proportion of $CD34^+$ $CD45^-$ cells in fetal lung. Together with other markers, these cells were identified as MSC. When these cells were expanded, the phenotype and differentiation potential was similar to that reported for MSC derived from adult bone marrow. As a model system for human hematopoiesis, Fibbe's group transplanted NOD/SCID mice with human $CD34^+$ cells derived from cord blood. When fetal MSC were cotransplanted with fetal cord blood-derived $CD34^+$ cells, the engraftment at 6 weeks was significantly enhanced, especially when low numbers of $CD34^+$ cells were present.

The engraftment promoting effect was specific for fetal MSC, and was significantly higher than observed if irradiated $CD34^-$ cells were co-transplanted. The engraftment promoting effect may not require the homing of MSC to the bone marrow, since following intravenous injection these cells were found primarily in the lung.

Robert Oostendorp (Rotterdam, The Netherlands) presented recently published data on stromal cell lines established from mouse aorto-gonad-mesonephros (AGM) subregions.[4] This region generates the first adult hematopoietic stem cells in the mouse embryo. Initially, the stem cell activity is localized to the dorsal aorta and mesenchyme (AM) and vitelline and umbilical arteries. Later, hematopoietic stem cell activity is found in urogenital ridges (UG), yolk sac, and liver. It is thought that these sites provide the supportive microenvironment until the bone marrow microenvironment is established. To better understand the cells and molecules involved in hematopoietic support in the midgestation embryo, numerous stromal cell lines and clones were established from the different sites. Using a marker gene it was found that both the AM and UG subregions produce many supportive clones, while the highest hematopoietic stem cell supportive clones were derived from the UGs. Interestingly, the liver at this stage yielded only few supportive clones.

Anthony Ho (Heidelberg, Germany) gave a lecture on the differentiation and the plasticity of hematopoietic stem cells (old cells learning new tricks). He summarized current knowledge about the origin of stem cells and the different reports of stem cells differentiating into other tissue types (trans-differentiation). Prerequisites for trans-differentiation are: (1) an initial demand for regeneration of the corresponding cell type must exist; (2) adult stem cells are able to migrate or 'home' on their own accord to the tissues where the demand arises; and (3) once settled in a niche after homing, surrounding cells in the microenvironment play a major role in defining the long-term fate of an adult stem cell. They seem then to be 'educated' by the neighboring cells to differentiate along the pathway of the surrounding cells and to maintain a balance between self-renewal and differentiation. The microenvironment plays a major role in the fundamental decision to self-renew or differentiate. The division of stem cells proceeds asymmetrically which is enhanced by stromal cell contact. Ho's group followed the behavior of single stem cells with a time-lapse camera system. From these experiments it was shown that asymmetric divisions are associated with primitive stem cell function. Asymmetric divisions cannot be influenced by regulatory molecules, but are altered by contact with stromal cells. Cross-talk of stem cells probably occurs through contact junctions. The long-term fate of stem cells is determined by direct communication with the microenvironment.

Michaela Feuring-Buske (Munich, Germany) presented data on the characterization of normal and leukemic stem cells in patients with acute myelogenous leukemia (AML). Leukemic stem cells are rare progenitors that most often express a phenotype very similar to that on normal long-term culture-initiating cells and NOD-SCID mouse repopulating cells. However, subtle differences, for example in the expression of growth factor receptors, may be used to selectively eliminate leukaemic stem cells *in vitro* and *in vivo*.[5] In recent years, the 'side population' (SP) of hematopoietic stem cells has been recognized. This SP is defined by the efflux of a supravital dye (Hoechst 33342) and comprises early hematopoietic stem cells in the murine system. It could be demonstrated that this SP exists in AML patients more frequently than in normal adult BM and is more heterogeneous than in the murine system. However, the $CD34^+CD38^-$ subfraction of the SP was found to be significantly enriched for cytogenetically normal stem cells in patients with AML.[6] Other data show that cells of the side population have functional plasticity and express the ABC transporter Bcrp1/ABCG2. Both findings suggest that the $CD34^+CD38^-$ fraction of the SP describes ontogenetically earlier stem cell candidates. Recent work by Feuring-Buske *et al* focuses on the amplification of normal repopulating stem cells without expansion of differentiated progeny. Using the method of retroviral gene transfer in human cord blood cells the homeobox transcription factor HOXB4 could be identified as a candidate gene for the amplification of genetically modified human hematopoietic stem cells without alteration of the normal differentiation program.[7] Therefore, HOXB4 also seems to be an attractive candidate for the amplification of normal stem cell populations from patients with AML.

Josef Mautner (Munich, Germany) presented data on the serological identification of MHC class II-restricted minor histocompatibility antigens (mHC). He first summarized the current knowledge about mHC that are defined by $CD8^+$ T cell clones. Two groups of mHC are known so far. The first group is encoded by the Y chromosome (SMCY and UTY genes), the second group is encoded by autosomal genes (HA-1, HA-2, HB-1, HA-8). The role of mHC defined by $CD4^+$ T helper cells is not well understood, but may be important for graft-versus-host or graft-versus-leukemia reactions. Mautner and coworkers stimulated T cells with lymphoblastoid cell lines established from B cells before and after transplantation. The specificity of T cell clones was tested by cytokine production. In the second set of experiments, a serological approach was taken screening patient sera for binding with recombinant cDNA expression libraries. In these experiments, mHC specific $CD4^+$ T helper cell clones were generated recognizing at least four different antigens and 19 targets of the humoral immune response were identified (three polymorphic). The specificity of these responses is still under investigation.

Edus Warren (Seattle, WA, USA) presented data on T cell therapy targeting minor histocompatibility antigens (mHC) for the treatment of leukemia and renal cell carcinoma. In the setting of an allogeneic transplant, T cell responses against minor histocompatibility antigens may cause graft rejection or GVHD, but may also mediate beneficial graft-versus-leukemia or graft-versus-tumor responses. After an allogeneic transplant, donor-derived $CD4^+$ and $CD8^+$ T cell clones specific for recipient mHC can be isolated from most recipients. The mHC defined by $CD8^+$ class I MHC-restric-

ted CTL show distinct patterns of tissue distribution, with some antigens expressed in both hematopoietic and non-hematopoietic tissues, but others expressed selectively in hematopoietic tissues. Antigens expressed selectively in hematopoietic cells, including leukemic cells, represent potential targets for adoptive T cell therapy designed to enhance graft-versus-leukemia reactions without inducing or aggravating GVHD. The total number of distinct human mHC is likely to be over 100. Warren and his coworkers have initiated a phase I study of adoptive immunotherapy with recipient mHC-specific, class I MHC-restricted $CD8^+$ T cell clones. The clones are generated prospectively at the time of transplant and characterized for mHC specificity and class I MHC restriction. Sequencing of the TCR β chain VDJ region of each clone permits the design of PCR-based assays that facilitate monitoring of the *in vivo* persistence and migration of adoptively transferred CTL. After relapse, standard chemotherapy is given, followed by a series of CTL infusions given at an escalating dose. Data on two patients treated with this approach were presented. One patient with relapsed Ph^+ $p190^+$ acute lymphoblastic leukemia achieved a complete morphologic and cytogenetic remission with chemotherapy followed by T cell therapy, but developed an acute lung injury syndrome after a dose of 2.25×10^9 CTL that showed prompt and complete resolution with corticosteroid therapy. Analysis of bronchoalveolar lavage fluid obtained shortly after the toxicity suggested that the toxicity was most likely attributable to recognition of mHC in the lung by adoptively transferred CTL. The mHA peptide recognized by this HLA-A29-restricted clone was subsequently identified by cDNA expression cloning as a protein product of an alternative open reading frame of the *FLJ12910* mRNA. A second patient with MDS/RAEB who relapsed post transplant with AML was treated with chemotherapy followed by infusions of a CTL clone specific for an HLA-B8-restricted mHC. This patient received a cumulative dose of $>5 \times 10^9$ CTL and tolerated all of his infusions well, with no GVHD and minimal grade 0–1 toxicity. Extending these studies to patients with metastatic renal cell cancer (RCC), $CD8^+$ mHC-specific CTL clones were isolated from three patients after non-myeloablative stem cell transplant. Data were presented for an HLA-A2-restricted clone that recognizes a mHC present on HLA-$A2^+$ RCC cell lines, suggesting that CTL clones reactive with mHC expressed on RCC cells could be used to enhance graft-versus-tumor effects after allogeneic transplant for metastatic RCC.

Maurice Zauderer (Rochester, MN, USA) described a novel system based on vaccinia cDNA libraries to identify T cell target antigens. This technology allows for functional selection of genes that mediate the cessation of cell growth or cell death.[8] An application is the identification of molecular targets of cytotoxic T cells. Initially, murine tumors were studied. More recently, tumor antigens of human melanoma, colon and kidney cancer are being identified which can form the basis for development of broadly effective vaccines. This technology can also be applied to selection of human monoclonal antibodies with a desired specificity, affinity or function. Yet another application is construction of cDNA libraries from differentiating hematopoietic or musculoskeletal stem cells for expression in a stem cell population. This enables selection of genes that regulate stem cell differentiation even though it results in cessation of cell growth.

Yair Reisner (Rehovot, Israel) presented new insights into the megadose concept of stem cell transplants across major histocompatibility barriers. Despite a mismatch at three HLA loci, megadose haploidentical transplants can engraft durably. As a possible explanation, it was shown that human CD34-positive cells have veto-activity (cells which prevent the development of CTL to a specific antigen but not to a third-party antigen). Likewise, veto cells in mice were identified in the Sca-1(+) Lin(−) fraction which also induced a specific tolerance toward donor-type skin grafts.[9] Furthermore, by using a TCR transgenic mouse model, in which the transgene is directed against $H2^d$, it was demonstrated that addition of Sca-1 (+) Lin(−) cells to MLR cultures led to deletion by apoptosis within 30 h, of the transgenic CD8 T cells. Deletion was found when the stem cells were of $H2^d$ origin, but not of $H2^k$ origin, illustrating the specificity of the veto activity. In a new study, human $CD34^+$ cells were expanded *in vitro* and produced a marked veto activity. Following 10–14 days of culture under myeloid differentiation conditions, a 28–80-fold expansion of cells with veto activity was observed,[10] thus affording a new source of veto cells without GVH reactivity. Other studies investigated $CD8^+$ CTLs with veto activity. Upon a selective starvation of interleukin-2, anti-host alloreactive T cells are depleted, and the generated CTL line was shown to be depleted of GVH reactivity *in vivo*. These cells express Fas ligand (FasL). Blocking experiments with antiCD8 and the use of CTL's generated from FasL mutated mice show that both FasL and CD8 are required for the veto effect.[11] Human CTLs depleted of anti-host reactivity have now been prepared in large numbers and clinical studies in the context of reduced intensity conditioning, combining 'mega-dose' CD34 cells with veto CTLs are underway.

Bryon Johnson (Milwaukee, USA) reported on studies analyzing donor lymphocyte infusions (DLI) in murine BMT. Previous work had identified thymus-derived Thy1+ NK1.1-regulatory cells of donor origin that suppress GVH reactivity induced by DLI after BMT. A large part of the suppression is mediated by $CD4^+$ T cells. It was hypothesized that this effect is mediated by $CD4^+CD25^+$ regulatory T cells. $CD4^+CD25^+$ regulatory T cells exhibit potent immunosuppresive function, and they have been shown to play an important role in suppression of autoimmunity and induction of allograft tolerance. Since CD40 ligand–CD40 and CD28–B7 interactions have been implicated in the generation of $CD4^+CD25^+$ cells, Johnson and coworkers wanted to test whether these costimulatory pathways are involved in the generation of regulatory T cells, and whether $CD25^+$ cells are directly involved in the generation of regulatory cells in DLI models. To address these questions, genetically deficient (knockout) mice were used that lack CD40 ligand, CD28 or CD25. From these experiments, Johnson and coworkers found that CD40 ligand–CD40 interactions are not involved in the generation of regulatory

cells. $CD4^+CD25^+$ cells are important in the suppression of DLI-induced GVH reactivity, however other $Thy1^+$ T cells may also contribute. B7–CD28 interactions are critical for generation of all $Thy1^+$ regulatory T cells (manuscript in preparation). Current work in Johnson's laboratory aims at the expansion and adoptive transfer of allosuppressive $CD25^+$ cells.

Andreas Mackensen (Regensburg, Germany) presented *in vitro* and *in vivo* data on allogeneic graft-versus-tumor responses in patients with melanoma. It was shown that following allogeneic peripheral blood stem cell transplantation (PBSCT) donor T cells can induce potent graft-versus-tumor (GVT) effects in hematological malignancies and possibly solid tumors such as renal cell carcinoma. Two patients (27 and 30 years old) with metastatic melanoma received allogeneic PBSCT from an HLA-identical sibling donor after reduced intensity conditioning with fludarabine, carmustine and melphalan. One patient showed a delayed mixed response with complete regression of lymph node metastases but progressive liver metastasis at day +120 consistent with a GVT response. In order to generate tumor-reactive cytotoxic T lymphocytes (CTL) from the donor, peripheral blood mononuclear cells were stimulated with donor monocyte-derived dendritic cells (DC) loaded with either tumor lysate or apoptotic tumor cells from the patient. Mackensen and coworkers had previously shown that two weekly stimulation cycles with tumor-loaded DC followed by restimulation with irradiated tumor cells alone were optimal for induction of tumor-specific CTL responses *in vitro*.[12] Using these culture conditions, a marked increase of $CD8^+$ CTL was observed exhibiting a strong MHC class I-restricted cytotoxic activity against the patient's tumor without cross-reactivity against EBV-transformed B cells from the donor. Length pattern analysis of the complementary determining region 3 (CDR3) of the T-cell receptor (TCR) Vβ chain revealed expansion of oligoclonal CTL populations. Functional (Elispot) and phenotypic (CDR3 spectratyping) analysis of patients' T cells at different time-points after transplantation revealed an expansion of alloreactive T cells with a polyclonal TCR Vβ pattern. Taken together, these results suggest that clinical GVT responses against melanoma can be induced after allogeneic PBSCT, and that the generation and expansion of oligoclonal tumor-reactive donor CTL *in vitro* may be used for adoptive T cell transfer after allogeneic transplantation.

Anne Dickinson (Newcastle, UK) reported on the current status of studies involving cytokine gene polymorphism as a risk factor for graft-versus-host disease (GVHD) and transplant outcome. Previous studies established the importance of polymorphisms of TNF-alpha, interleukin-10, interferon-gamma, interleukin-1 receptor antagonist and interleukin-6 genes in predicting GVHD.[13] In a multicenter setting, the predictive values of cytokine gene polymorphisms were assessed for acute and chronic GVHD. A total of 242 sibling-matched transplants was evaluated. Generally, in pediatric centers no association with the described gene polymorphisms was found and increased GVHD prophylaxis appeared to alter the risk profile. Most associations remained valid for acute GVHD. After multivariate analysis, recipient interleukin-10 and recipient interleukin-6 remained as risk factors for developing chronic GVHD. The size of the risk was comparable to known clinical risk factors like age, diagnosis or sex mismatch.

James Ferrara (Ann Arbor, MI, USA) presented data which showed that acute graft-versus-host disease does not require alloantigen expression. Alloantigen expression on host antigen-presenting cells (APCs) is essential to initiate graft-versus-host disease (GVHD), and alloantigen expression on host target epithelium was therefore also thought to be essential for tissue damage. Ferrara and coworkers tested this assumption in mouse models of GVHD using bone marrow chimeras in which either MHC II or MHC I alloantigen was expressed only on APCs. They found that acute GVHD was equally severe in a $CD4^+$-mediated model when bm12 donors were transplanted into [B6 → B6] and [B6 → II −/−] recipients (the latter mice expressing MHC II alloantigens only on APCs). Similar results were found in a $CD8^+$ dependent model when bm1 donors were transplanted into [B6 → B6] and [B6 → bm1] recipients where epithelial targets were syngeneic to the donor and only the APCs of the recipient expressed alloantigen. In this model, clinical GVHD was less severe in recipients that expressed alloantigen only on APCs, but histologic GVHD was equally severe in both groups. In both the $CD4^+$- and $CD8^+$-mediated models neutralization of TNF alpha and IL-1 prevented both histologic and clinical acute GVHD (see Figure 2). Thus acute GVHD does not require alloantigen expression on host target epithelium and neutralization of inflammatory cytokines prevents acute GVHD. These results pertain particularly to CD4-mediated GVHD but also apply, at least in part, to CD8-mediated GVHD. Ferrara's data challenge current paradigms about the antigen specificity of GVHD effector mechanisms and confirm the central roles of both host APCs and inflammatory cytokines in acute GVHD.[14]

Clemens-Martin Wendtner (Munich, Germany) reported on new studies exploring gene therapy for chronic lymphocytic leukemia (CLL). Previous attempts at gene transfer were hampered by low transduction efficiency and lack of target specificity. Recombinant EBV-derived vectors were studied *in vitro* in 14 patients with B-CLL and achieved a transduction efficiency of up to 85%. In another series of experiments, recombinant adeno-associated virus (AAV) vectors (coding for enhanced green fluorescent protein and CD40 ligand) were packaged in an adenovirus-free system and achieved high genomic titres. These vectors were studied *in vitro* in 24 patients with B-CLL, resulting in transgene expression of up to 97%. Transduction with AAV/CD40 ligand resulted in up-regulation of the costimulatory molecule CD80 not only on infected, but also on bystander leukemic CLL cells which induced a specific allogeneic T cell response.[15] Both approaches for gene transfer appear promising for the development of CLL-specific vaccines.

John Barrett (Bethesda, MD, USA) in his lecture addressed the question as to how bone marrow transplantation can

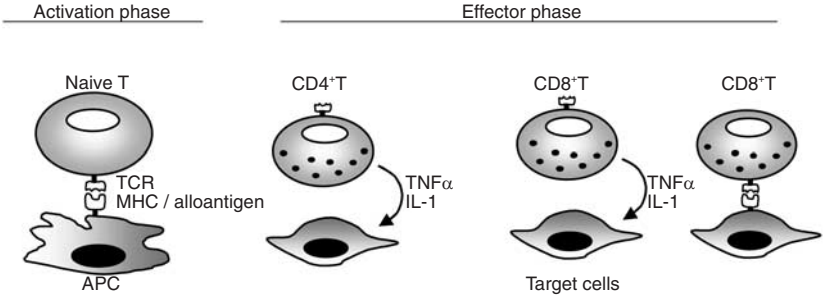

Figure 2 Interaction of T cells with antigen-presenting cells (APC) and target cells of GVHD showing in a mouse model that alloantigen on host epithelium is not required for acute GVHD and that the neutralization of inflammatory cytokines can prevent acute GVHD.

be improved by a selective manipulation of the immune repertoire. One way of improving the balance between toxicity, GVHD, immune dysfunction on one side and antitumor effects on the other side, would be to selectively deplete alloreactive T cell from the graft. This approach is attractive since recipient lymphocytes express both ubiquitous and tissue-restricted minor histocompatibility antigens and bear a significant portion of the GVHD message. Previous studies showed that a selective depletion preserves third-party responses, anti-viral responses and anti-leukemic responses.[16,17] Barrett presented data on two new approaches in which pure expanded T cells are used as stimulators of alloresponses. Activated cells are then removed by incubation with an immunotoxin directed against CD25. In the first study, older patients who have at least 50% risk of grade II–IV GVHD are being treated in the context of a reduced-intensity conditioning with fludarabine, cyclophosphamide and a short course of cyclosporin A. So far two patients with myelodysplastic syndromes were transplanted. These patients had no major toxicity and obtained at short follow-up a 100% donor T cell and a mixed myeloid chimerism. The second study applies the concept of selective depletion to mismatched or pseudo-haploidentical transplantations. *In vitro*, mismatched CTL exerted a powerful inhibition of leukemic colony growth. CTL stimulated by leukemic cells showed lineage-restricted activity. In such cultures, the T cell receptor Vβ repertoire skewing was also lineage-specific.

Thomas Brocker (Munich, Germany) reported his work on transgenic mice and dendritic cells *in vivo*. Dendritic cells are professional antigen presenting cells which can induce immune responses by activating naive antigen-specific T cells directly. Dendritic cells are present in the thymic medulla and appear to be important in negative selection, but not positive selection of T cells. In order to delineate the function of dendritic cells *in vivo*, Brocker and his coworkers generated transgenic mice expressing various molecules specifically in dendritic cells, so that DC-functions in MHC class I- or MHC class II-restricted T cell responses can be followed *in vivo*. In these mice, the function of DC can be compared with B cell function, as well as effects of DC on natural killer-cell education can be studied. These studies offer new insights into immunoregulation and potentially new approaches to immunotherapy with dendritic cells.

Matthias Theobald (Mainz, Germany) presented data on preclinical models aimed at enhancing T cell immunity to cancer- or leukemia-associated antigens and viruses like CMV. The concept of modulating T cell-mediated immunity is theoretically attractive for the transplantation of hematopoietic stem cells. T cells mediating graft-versus-host disease may be deleted or tolerized, whereas cytotoxic T cells recognizing leukemia-associated or virus-specific antigens may be preserved or expanded. Theobald's group equipped recipient-derived T lymphocytes with 'off-the shelf' available T cell antigen receptors (TCRs) specific for leukemia-associated, as well as human cytomegalovirus-specific antigenic epitopes, in order to tackle leukemic relapse and CMV infection. This strategy is attractive as immunotherapy of malignant disease by transferring specificity and affinity of TCRs for broad-spectrum tumor- and leukemia-associated antigens into T lymphocytes of patients and thus breaking their state of cancer- and leukemia-specific T cell tolerance. Mice transgenic for HLA-A*0201 were developed to bypass the obstacles of CTL-based therapies like self-tolerance, low avidity or maintenance of antigen specificity. As an example, a CTL target epitope derived from the human MDM2 oncoprotein was identified. In A*0201 transgenic mice self-tolerance was bypassed, and A*0201 negative, allorestricted human T cells were generated. High-affinity transgenic mouse and allogeneic human CTLs specific for the A*0201-presented epitope specifically killed a broad range of malignant targets. Further, the murine TCRs were partially humanized by transferring constant regions of human origin and thereby diminishing the risk of a potential immune response.[18]

B Eiz-Vesper (Hannover, Germany) reported on the V119I polymorphism of proteinase-3. The frequency of this polymorphism was determined in 221 healthy blood donors and 104 donor/recipient pairs of stem cell transplants (V/I, 0.56/0.44). CTLs from donors were stimulated with proteinase-3-deduced peptides covering the V119I variation. Decamers were chosen according to the binding motif for HLA-A*0201. In cases of proteinase-3 discordant donor–recipient pairs, significant responses were observed. These data provide evidence that proteinase-3 may act as a mHC.

Ulrich Steidl (Düsseldorf, Germany) presented his recently published data on gene expression profiling of CD34$^+$ progenitors from bone marrow and peripheral blood. Mobilized peripheral stem cells are widely used in autologous and allogeneic transplantation, however, the complete gene

expression profile of human hematopoietic stem cells has not been investigated so far. The authors purified stem cells from 10 samples of healthy donors mobilized by G-CSF and compared the gene expression to eight samples of bone marrow-derived $CD34^+$ cells. Bone marrow-derived stem cells had a higher expression of several cell cycle- and DNA-synthesis-related genes, whereas mobilized stem cells had a higher expression of five apoptosis-related genes. By cluster analysis, both types of stem cells could be clearly segregated. The gene expression profile molecularly explains previous data that peripheral stem cells are more quiescent than bone marrow stem cells and may give new insights into the physiology of human stem cells.[19]

Harry Dolstra (Nijmegen, The Netherlands) reported new studies on the mHC HB-1. The molecular nature of HB-1 was identified through cDNA expression cloning.[20] HB-1 is expressed in two allelic variants, that differ in a single amino acid, thereby influencing T cell receptor recognition. The transcript for HB-1 is differentially expressed in all samples of B-type acute lymphoblastic leukemia, a subset of B follicular lymphoma, EBV-transformed B lymphoid cells, but not in normal cells and tissues. Using monocyte-derived dendritic cells pulsed with peptides or the full-length protein, both $CD4^+$ and $CD8^+$ T cell clones were generated against HB-1-derived epitopes. HB-1 has the potential as a vaccine target to boost GVL reactivity and for autologous immunotherapy, eg in the situation of minimal residual disease after primary therapy.

Anthony Dodi (The Anthony Nolan Institute, London, UK) presented data related to new immunotherapeutic approaches to bcr-abl-positive chronic myelogenous leukemia (CML). K562 cells (bcr-abl positive, but negative for HLA class I antigens) were transfected with single HLA-A3 and HLA-B8 antigens. CML-specific peptides were eluted from their surface and their sequences defined. Using these peptides tetramers were then produced. It was demonstrated for the first time that patients with CML express HLA-associated leukemia-specific peptides from the bcr-abl translocation and have circulating CTLs specific for the bcr-abl fusion peptides and that these cells can be expanded with specific peptides *in vitro*. *In vitro* peptide-stimulated cells show specific killing of autologous leukemic targets and of HLA-matched leukemic cells, but not of HLA-mismatched leukemic target cells. These findings provide the basis for an immunotherapeutic approach to CML.[21,22]

Xiao-nong Wang (Newcastle, UK) presented data on the *in situ* localization (skin explant assay) of CTLs specific for the ubiquitously expressed mHag H-Y and the hematopoietic system-restricted mHCs HA-1 and HA-2. mHC H-Y-specific CTLs induced severe (grade III–IV) GVH reactions *in situ*, whereas CTLs specific for mHC HA-1 or HA-2 induced low or no GVH reactivity (grade I–II) unless the skin section was preincubated with HA-1 or HA-2 synthetic peptides. These data provide evidence that GVH and GVL reactivity can be separated based on the type and tissue distribution of mHCs recognized.[23]

Julia Winkler (Erlangen, Germany) analyzed B lymphopoiesis after allogeneic transplantation and compared recipients of bone marrow and mobilized peripheral stem cells. In both groups of patients, an active donor-derived B-lymphopoiesis with comparable numbers of $CD19^+CD10^+$ pre-B cells was found. These data provide evidence that peripheral stem cells provide long-term B lymphoid engraftment.

James Ferrara (Ann Arbor, MI, USA), *John Goldman* (London, UK) and a panel of experts summarized the findings of the symposium. The focus of the symposium was on the basic mechanisms underlying graft-versus-host disease and graft-versus-leukemia reactions. Progress has been made in the characterization of stem cells, as well as of other cells like veto cells, mesenchymal cells, and regulatory T cells. Minor histocompatibility antigens, targets of cytotoxic T lymphocytes and the role of cytokine–cell interactions have all been defined in more detail. The inhibition of alloreactions may also be reached by several means (removal of antigen-presenting cells, neutralization of inflammatory cytokines, costimulatory blockade and even other mechanisms). At this moment, gene therapy and the selective depletion of cytotoxic T lymphocytes have begun to enter clinical bone marrow transplantation. Gene chip technology will provide new information and expand the classification of malignancies and stem cell subpopulations. Problems remain and bone marrow transplantation will have to compete with receptor-based therapies which have already been introduced into the clinical setting in CML for example. The immune system uses multiple circuits in its effector mechanisms (network theory of the immune system). This is certainly also true for graft-versus-leukemia reactions. As mentioned by several speakers at the meeting, tumor cells use multiple mechanisms of immune evasion. Goldman concluded therefore not a single treatment, but a combination of several approaches will be necessary to reach the goal of cure in all patients. In the future bone marrow transplantation is likely to advance toward a selective component therapy, in order to become a less toxic and more effective treatment for a broader range of patients.

References

1 Rossig C, Bollard CM, Nuchtern JG *et al*. Epstein–Barr virus-specific human T lymphocytes expressing antitumor chimeric T cell receptors: potential for improved immunotherapy. *Blood* 2002; **99**: 2009–2016.
2 Horwitz EM, Prockop DJ, Gordon PL *et al*. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood* 2001; **97**: 1227–1231.
3 Li Z, Düllmann J, Schiedlmeier B *et al*. Murine leukemia induced by retroviral gene marking. *Science* 2002; **296**: 497.
4 Oostendorp RA, Harvey KN, Kusadasi N *et al*. Stromal cell lines from mouse aorto-gonads-mesonephros subregions are potent supporters of hematopoietic stem cell activity. *Blood* 2002; **99**: 1183–1189.
5 Feuring-Buske M, Frankel AE, Alexander RL *et al*. A diphtheria toxin-interleukin 3 fusion protein is cytotoxic to primitive acute myeloid leukemia progenitors but spares normal progenitors. *Cancer Res* 2002; **62**: 1730–1736.
6 Feuring-Buske, Hogge DE. Hoechst 33342 efflux identifies a

subpopulation of cytogenetically normal $CD34^+CD38^-$ progenitor cells from patients with acute myeloid leukemia. *Blood* 2002; **97**: 3882–3889.
7 Buske C, Feuring-Buske M, Abramovich C *et al*. Deregulated expression of HOXB4 enhances the primitive growth activity of human hematopoietic cells. *Blood* 2002; **100**: 862–868.
8 Smith ES, Mandokhot A, Evans EE *et al*. Lethality based selection of recombinant genes in mammalian cells: application to identifying tumour antigens. *Nat Med* 2001; **7**: 967–972.
9 Bachar-Lustig E, Li HW, Gur H *et al*. Induction of donor-type chimerism and transplantation tolerance across major histocompatibility barriers in sublethally irradiated mice by Sca-1 (+) Lin (−) bone marrow progenitor cells: synergism with non-alloreactive (host x donor) F (1) T cells. *Blood* 1999; **94**: 3212–3221.
10 Gur H, Krauthgamer R, Berrebi A *et al*. Tolerance induction by megadose hematopoietic progenitor cells: expansion of veto cells by short term expansion of purified human CD34 cells. *Blood* 2002; **99**: 4174–4181.
11 Reich-Zeliger S, Zhao Y, Krauthgamer R *et al*. Anti-third party CD8 CTLs as potent veto cells: coexpression of CD8 and FasL is a prerequisite. *Immunity* 2000; **13**: 507–515.
12 Kurokawa T, Oelke M, Mackensen A. Induction and clonal expansion of tumor specific cytotoxic T lymphocytes from renal cell carcinoma patients after stimulation with autologous dendritic cells loaded with tumor cells. *Int J Cancer* 2001; **91**: 749–756.
13 Dickinson AM, Cavet J, Cullup H *et al*. GVHD risk assessment in hematopoietic stem cell transplantation: role of cytokine gene polymorphisms and an in vitro human skin explant model. *Hum Immunol* 2001: **62**; 1266–1276.
14 Teshima T, Ordemann R, Reddy P *et al*. Acute graft-versus host disease does not require alloantigen expression on host epithelium. *Nat Med* 2002; **8**: 575–581.
15 Wendtner CM, Kofler DM, Theiss HD *et al*. Efficient gene transfer of CD40 ligand into primary B-CLL cells using recombinant adeno-associated virus (rAAV) vectors. *Blood* 2002; **100**: 1655–1661.
16 Mavroudis DA, Dermine S, Molldrem J *et al*. Specific depletion of alloreactive T cells in graft-versus-host and graft-versus-leukaemia reactions. *Br J Haematol* 1998; **101**: 565–570.
17 Cavazzana-Calvo M, Andre-Schmutz I, Hacein-Bey-Abina S *et al*. Improving immune reconstitution while preventing graft-versus-host disease in allogeneic stem cell transplantation. *Semin Hematol* 2002; **39**: 32–40.
18 Stanislawski T, Voss RH, Lotz C *et al*. Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer. *Nat Immunol* 2001; **2**: 962–970.
19 Steidl U, Kronenwett R, Rohr UP *et al*. Gene expression profiling identifies significant differences between the molecular phenotypes of bone marrow-derived and circulating human $CD34^+$ hematopoietic stem cells. *Blood* 2002; **99**: 2037–2044.
20 Dolstra H, Fredrix H, Maas F *et al*. A human minor histocompatibility antigen specific for B cell acute lymphoblastic leukemia. *J Exp Med* 1999; **189**: 301–308.
21 Clark RE, Dodi IA, Hill SC *et al*. Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. *Blood* 2001; **98**: 2887–2893.
22 Norbury LC, Clark RE, Christmas SE. b3a2 BCR-ABL fusion peptides as targets for cytotoxic T cells in chronic myeloid leukaemia. *Br J Haematol* 2000; **109**: 616–621.
23 Dickinson AM, Wang XN, Sviland L *et al*. *In situ* disection of the graft-versus-host activities of cytotoxic T cells specific for minor histocompatibility antigens. *Nat Med* 2002; **8**: 410–414.