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Graft vs. Leukaemia Reactions in Chronic Myeloid Leukaemia

Jonathan O. Cullis, A. John Barrett and John M. Goldman

A 39-year-old female relapsed 36 months after allogeneic bone marrow transplantation for chronic myeloid leukaemia. Infusion of peripheral blood leucocytes from her bone marrow donor resulted in complete remission, and she remains leukaemia-free 18 months later. This case provides direct evidence for a 'graft vs. leukaemia' (GVL) effect contributing to the eradication of leukaemia after marrow transplantation. Existing evidence for GVL and its possible mechanisms are reviewed.
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INITIAL PRESENTATION AND MANAGEMENT

A 39-YEAR-OLD female architect first presented in November 1984 with complaints of hair loss and fatigue. A full blood count, performed by her general practitioner to exclude iron deficiency, showed a haemoglobin of 10.1 g/dl, white blood cell count of 134 × 10^9/l and platelet count of 435 × 10^9/l. The differential showed 1% promyelocytes, 12% myelocytes, 3% metamyelocytes, 67% neutrophils, 9% lymphocytes, 1% monocytes, 2% eosinophils and 5% basophils [1]. She was referred to the local haematologist.

On clinical examination, the spleen was just palpable, but there were no other abnormal findings. A bone marrow aspirate showed marked hypercellularity with granulocytic hyperplasia consistent with a diagnosis of chronic myeloid leukaemia (CML) in chronic phase. The diagnosis was confirmed by cytogenetic analysis which revealed the presence of a complex Philadelphia

chromosome translocation in all metaphases examined, karyotype 46,XX,t(9;21;22) (q34;p1;q11), del(3) (p13p21).

The patient's initial management comprised oral chemotherapy with intermittent busulphan, but in view of her young age, she was considered for allogeneic bone marrow transplantation (BMT). Tissue typing revealed HLA-A, B and DR identity between her and one of her two brothers.

ALLOGENEIC BONE MARROW TRANSPLANT

She proceeded to undergo allogeneic BMT at Hammersmith Hospital in February 1986, while still in the chronic phase of CML. The pre-transplant conditioning comprised cyclophosphamide 120 mg/kg, daunorubicin 60 mg/m^2, fractionated total body irradiation (TBI) 12 Gy and splenic irradiation 10 Gy. The donor marrow was depleted of T-lymphocytes *in vitro* using Campath 1M [2]. No additional graft vs. host-disease (GVHD) prophylaxis was given.

The initial post-transplant course was complicated by the development of grade II acute GVHD of skin and gut on day 14, but this settled rapidly on high-dose intravenous steroids, and she was fit for discharge by day 27. Her steroid dosage was tapered rapidly to zero. She remained well until June 1986, when she developed a scaly skin rash, nausea, vomiting and diarrhoea. Biopsies of skin and gastric mucosa showed changes consistent with chronic GVHD, and steroids were reintroduced with good effect. However, her symptoms recurred on reducing the steroid dosage and cyclosporin A (CSA) was introduced.

She was re-admitted in February 1987, 12 months post-BMT, when she developed a dry cough and left upper lobe shadowing on chest X-ray. Extensive investigations, including bronchoalveolar lavage, failed to reveal a pathogen, but there was a clinical response to amphotericin B. In view of the presumed fungal infection, steroids were discontinued, but within a month her skin GVHD had flared, and PUVA (psoralens plus ultraviolet radiation A) therapy was commenced. Her skin gradually improved over the subsequent months.

In June 1987 she again developed nausea and vomiting, but on this occasion, there was no evidence of GVHD on gastric biopsy, and her symptoms settled on discontinuation of CSA.

SUBSEQUENT FOLLOW-UP

Routine bone marrow aspirates and cytogenetic analyses were performed at regular intervals in the post-BMT period. The cytogenetic results are shown in Table 1. The patterns of cytogenetic results seen post-BMT for chronic phase CML are complex: the transient detection of Philadelphia chromosome-positive metaphases with subsequent return to completely Philadelphia-negative haematopoiesis is well recognised [3–7] (transient cytogenetic relapse); a solitary Philadelphia-positive metaphase was detected in this patient at 6 months. Sustained Philadelphia-positive haematopoiesis, however, carries a very high risk of progression to overt clinical relapse. The detection of a further solitary Philadelphia-positive metaphase in this patient at 36 months post-BMT was followed by a steady increase in the proportion of Philadelphia-positive cells in the bone marrow to 83% by November 1990, 57 months post-BMT. At this stage, the patient was extremely well, and had no other clinical or laboratory evidence of CML. Cytogenetic analysis of her phytohaemagglutinin- and pokeweed mitogen-stimulated peripheral blood lymphocytes revealed these to be of donor origin.

Table 1. Marrow cytogenetic results after BMT

Months post-BMT	No. Ph-positive metaphases/total analysed (%)
3	0/20 (0)
6	1/15 (7)
9	0/15 (0)
12	0/10 (0)
19	0/10 (0)
29	0/10 (0)
36	1/18 (6)
48	6/35 (17)
54	18/30 (60)
57	29/35 (83)

MANAGEMENT OF CML IN RELAPSE FOLLOWING BMT

The optimal management of patients in relapse after BMT for CML is uncertain. Conventional chemotherapy with busulphan or hydroxyurea is unlikely to prolong life, although the disease may behave in relatively indolent fashion [5]. α-Interferon (IFN) may suppress the Philadelphia-positive clone in a minority of patients [8]. Although in general second bone marrow transplants carry a high morbidity and mortality [9], we have recently described encouraging results in patients conditioned with high-dose busulphan alone [10]. More recently, the use of donor leucocyte transfusions has been reported in this situation. Kolb *et al.* [11] treated 3 patients in haematological relapse following BMT with IFN and infusions of buffy coat cells from the original marrow donor: all 3 became Philadelphia-negative within 18 weeks of this therapy and remained so at 32–91 weeks follow-up. 2 of the 3 developed moderate GVHD. On the basis of this report, we opted to treat this patient by a similar approach, but without IFN. She, therefore, received a single infusion of buffy coat cells, derived from her HLA-identical donor brother by leukapheresis, in November 1990. The total nucleated cell dose infused was 1.75×10^8/kg. Because of concerns about her previous GVHD, she was given methotrexate 8 mg/m^2 intravenously on days 2, 4, 8 and 12, and CSA 5 mg/kg daily by mouth.

Two months after the buffy coat transfusion, her marrow metaphases remained substantially Philadelphia-positive (70% of metaphases examined, Fig. 1) and CSA was discontinued. 2 months later she developed an itchy skin rash and oral ulceration. A clinical diagnosis of GVHD was made, and her symptoms settled rapidly on oral steroids. A further marrow aspirate performed at this stage showed 100% donor-type haematopoiesis with no Philadelphia-positive metaphases detectable (Fig. 1). At most recent follow-up, she remains completely well and in complete haematological and cytogenetic remission.

POLYMERASE CHAIN REACTION AND MINIMAL RESIDUAL DISEASE AFTER BMT FOR CML

A more sensitive method than cytogenetics for identifying residual leukaemic cells post-BMT for CML is the polymerase

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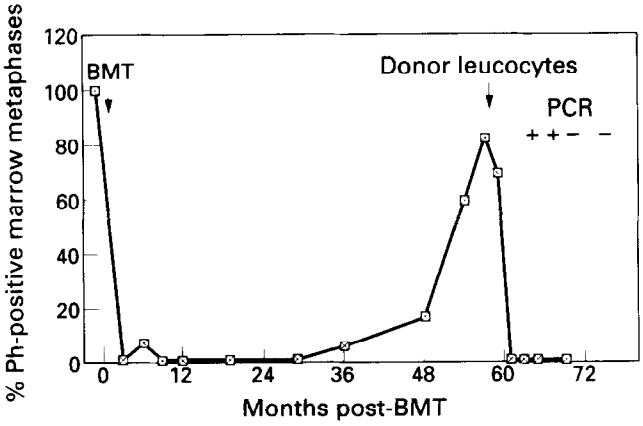

Fig. 1. Cytogenetic and PCR results in the patient before and after infusions of donor leucocytes.

chain reaction (PCR) [12–18]. The Philadelphia translocation results in the formation of a unique chimeric gene, BCR/ABL, by juxtaposition of sequences from the BCR gene on chromosome 22 with the bulk of the ABL gene from chromosome 9. The BCR/ABL gene encodes a 210 kD protein with tyrosine kinase activity, $P210^{BCR/ABL}$, believed to be critical in leukaemic transformation. It is theoretically possible to use PCR to amplify DNA across the fusion point on the chimeric gene, but for practical reasons it is easier to amplify the BCR/ABL mRNA. This technique is capable of detecting one leukaemic cell in 10^5 to 10^6 normal cells, and appears to have some value in the prediction of relapse post-BMT [19]. BCR/ABL transcripts are frequently detectable by PCR within the first 6–9 months post-BMT, even in patients not destined to relapse, but patients in whom these transcripts are still detectable after this time appear to be at increased risk of relapse. As shown in Fig. 1, the patient we have described had detectable BCR/ABL mRNA 3 and 6 months after the infusion of donor leucocytes, but by 9 months this had become undetectable, and remains so at 15 months. It is, therefore, highly likely that she has been cured of CML.

GRAFT VS. LEUKAEMIA (GVL) EFFECT

The case described adds further evidence in support of what has become known as the graft vs. leukaemia effect. It has long been recognised that immune mechanisms are important in the eradication of leukaemia after BMT. As early as 1956, Barnes *et al.* [20], working with murine BMT, postulated that allogeneic marrow might be capable of eradicating leukaemia which was not eliminated by conditioning therapy. A substantial body of experimental and clinical data now supports this hypothesis.

In man, the likelihood of leukaemic relapse after BMT is influenced by several factors: relapse occurs more commonly after syngeneic BMT than after allogeneic BMT [21], whereas GVHD is associated with a decreased risk of leukaemic relapse [22]. Attempts to abolish GVHD by T-cell depletion of donor marrow have led to an increased risk of relapse [23–26]. GVHD, therefore, seems to have an antileukaemic effect, and the occasional observations of patients in relapse returning to complete remission during exacerbations of GVHD or on discontinuation of immunosuppression are consistent with this idea [27, 28]. However, it appears that other mechanisms, independent of GVHD, may also be operative in the eradication of leukaemia post-BMT. A large retrospective study by Horowitz *et al.* [25] of over 2000 patients reported to the International Bone Marrow Transplant Registry (IBMTR) examined the influence of GVHD and T-cell depletion on relapse following BMT for leukaemia. Decreased risk of relapse was seen in patients with GVHD. Subgroup analysis for acute myeloid leukaemia (AML) patients showed an increased risk of relapse in patients receiving syngeneic marrow compared with those receiving allogeneic marrow without GVHD, suggesting an antileukaemic effect of allografts independent of GVHD. For patients with CML, relapse risk was significantly increased in recipients of T-cell depleted marrow, and this effect remained significant after exclusion of GVHD as a confounding variable. Taken together, these data have been interpreted as evidence that GVL and GVHD effects are distinct, and highlight the important role of donor T-cells in the eradication of leukaemia, especially in CML [29].

MECHANISMS OF THE GVL EFFECT

There is evidence that several different antileukaemic mechanisms are operative following BMT, and it is convenient to divide these into major histocompatibility complex (MHC)-unrestricted and MHC-restricted mechanisms.

MHC unrestricted mechanisms

Natural killer (NK) cells, morphologically large granular lymphocytes, which are CD3−, CD16+, CD56+, and lack α/β or γ/δ T-cell receptor gene rearrangement, are capable of exerting powerful inhibitory effects on normal haemopoiesis [30, 31], and can lyse leukaemic cell lines [32] and fresh leukaemic cells [33] without prior exposure to antigen. Cytotoxic effects are mediated either via the release of cytotoxic molecules from the NK cell granules at the effector–target cell interface, or indirectly via the effects of cytokines such as α-IFN, γ-IFN, and tumour necrosis factor-α (TNF-α), and cytotoxicity is enhanced by exposure to interleukin-2 (IL-2). NK cells are prominent in the blood of patients after BMT, and the generation of lymphokine-activated killer (LAK) cells is increased [34]. NK cells cytotoxic to a patient's cryopreserved acute lymphoblastic leukaemia (ALL) cells have been isolated post-BMT [35], and LAK cells derived from the blood of patients post-BMT for CML were shown to exhibit cytotoxicity to the patients' cryopreserved CML cells, and to inhibit the patients' leukaemic CFU-GM, but not donor-derived CFU-GM [36].

MHC restricted mechanisms

Experiments in both mice [37, 38] and man have demonstrated that both CD4+ and CD8+ T-lymphocyte subsets are capable of exerting GVL effects. In man, both CD4+ and CD8+ alloreactive cytotoxic T-cell (CTL) clones with antileukaemic activity have been generated. Sosman *et al.* [39] used leukaemic cells from a patient with ALL to stimulate T-cell responses in unrelated individuals. Although the majority of clones thus generated would recognise both the patient's leukaemic cells and non-leukaemic remission lymphocytes, a small proportion would only recognise and lyse the leukaemic cells, and were thus 'leukaemia-specific'. These clones were CD4+ and MHC class II restricted. Similar experiments in our department [40] have demonstrated that CD4+, MHC class II restricted CTL clones can be generated against CML targets.

Faber *et al.* [41] used leukaemic cells from 1 patient with AML and another with CML to generate T-cell clones from HLA-identical siblings. Among the clones generated for the patient with AML were CTL clones capable of lysing patient leukaemic cells but not patient lymphocytes or Epstein–Barr virus (EBV) transformed lymphocytes: these clones were CD8+ and MHC class I restricted. In the CML patient, no leukaemia-

specific clones were generated, but alloreactive CD8+, HLA-A2 restricted CTL clones, capable of lysing patient leukaemia cells, lymphocytes and EBV-transformed lymphocytes were found.

The fact that antileukaemic CTL clones can be generated *in vitro* raises the important question of what antigens these T-cells might recognise. It has become apparent in recent years that MHC molecules present antigen to T-lymphocytes in the form of short peptide sequences [42, 43]. MHC class I molecules largely present peptides derived from endogenous cellular proteins, and interact with CD8+ T-cells, whereas class II molecules are largely concerned with the presentation of exogenous antigens to CD4+ T-cells (44–46]. Because responses to self-peptides are clonally deleted in the thymus during lymphocyte ontogeny, subsequent T-cell responses are directed to exogenous antigens or abnormal cellular antigens, such as virally encoded proteins or tumour-specific antigens.

Following HLA-identical BMT, lymphocytes recognise differences in the self-peptides presented in the context of MHC molecules: these minor histocompatibility antigens (mHA) thus form the basis of graft rejection [47] and GVHD [48], and it is possible that they also provide a basis for GVL. Preferential expression of a mHA by the leukaemic clone might thus enable a leukaemia-selective response by donor-derived T-cells of appropriate specificity, whereas, if expression of the mHA was more widespread, a less selective response might result, with accompanying GVHD.

A true leukaemia-specific T-cell response might occur if leukaemia-specific antigens were presented via MHC molecules. The BCR/ABL proteins in CML and other Philadelphia chromosome-positive leukaemias are composed of normal N-terminus BCR sequences and C-terminus ABL sequences [49]: MHC-bound peptides from these sequences would not be expected to elicit T-cell responses as they are self-peptides. However, the BCR/ABL junctional region provides a unique determinant that

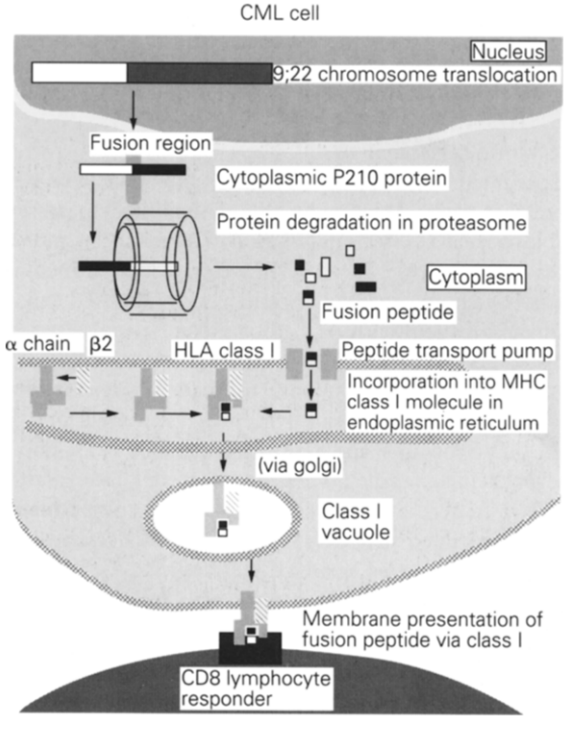

Fig. 2. Mechanism for possible presentation of BCR/ABL fusion peptides via MHC class I molecules to a CD8+ lymphocyte [46].

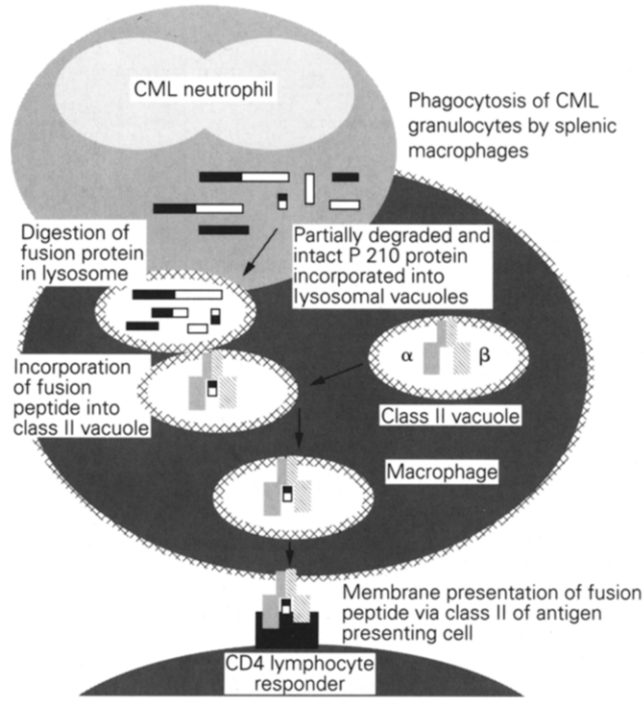

Fig. 3. Possible mechanism for MHC class II presentation of BCR/ABL fusion peptides to a CD4+ lymphocyte.

is not present in normal cells and might prove to be antigenic if presented in the context of MHC molecules, as shown diagrammatically in Figs 2 and 3. Only limited data have so far been obtained in man on the ability of BCR/ABL junctional peptides to elicit T-cell responses [50], but Chen *et al.* [51] have demonstrated that a BCR/ABL peptide injected into mice elicited CD4+, MHC class II restricted T-cell responses. The junctional sequence from another leukaemia-specific chimeric protein, MYL/RAR-α, formed as a result of the t(15;17) translocation characteristic of acute promyelocytic leukaemia, has been shown to be capable of eliciting human T-cell responses *in vitro* [52].

FUTURE DIRECTIONS

Clearly, the idea that leukaemia-specific antigens might exist is very attractive, since they could provide a means for separating the GVL and GVHD responses in a clinically useful way. Attempts in man to enhance GVL without increasing GVHD have thus far proved unsuccessful: Sullivan *et al.* [53] gave patients with advanced leukaemia donor leucocyte infusions post-BMT in an attempt to reduce relapse risk, but these patients developed more GVHD and had higher mortality than control patients. Attempts to selectively deplete T-cell subsets likely to cause GVHD from the donor marrow, without removing the effectors of GVL seem unlikely to succeed using existing techniques, since the cells mediating the two effects are probably similar, although it is of interest that a recent report from the IBMTR [26] suggests that the use of monoclonal antibodies to deplete donor marrow of T-cells is associated with a higher risk of subsequent relapse than physical methods of T-cell depletion. Work in our department has recently demonstrated that it is possible to clonally delete donor T-cell responses against recipient lymphoblasts *in vitro*, while preserving responses to recipient CML cells [54], although it remains to be seen whether this technique has clinical applications.

The administration of IL-2 or LAK cells to allogeneic BMT

patients has not been extensively investigated [55], although murine experiments suggest IL-2 might enhance GVL without affecting GVHD [56]. A potentially exciting development is the recent observation that T-cell clones directed against cytomegalovirus (CMV) epitopes could restore anti-CMV immunity to BMT recipients [57], suggesting that it may be possible to raise T-cell clones specific for leukaemia antigens, which could then be infused into patients post-BMT to reduce the risk of relapse, or used in a manner similar to donor leucocyte infusions to treat patients in relapse following BMT.

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Classification of Hodgkin's Disease: Yesterday, Today and Tomorrow

John E. Ultmann

THIS BRIEF review of staging classifications for Hodgkin's disease will trace the development of various classifications over the nearly 100 years since a clinical classification was devised by Dorothy Reed in 1902 [1]. Classifications are an attempt to predict outcome of disease as affected by its extent, the current philosophies of pathophysiology of spread, and of course, the treatment modalities available.

When Reed developed a staging classification in 1902, no treatment was available and the classification simply addressed the fact that there were two stages, localised disease and advanced disease; the former usually preceded the latter. The strategy for identification of risk factors was largely unknown; the aim of treatment was palliation; treatment in fact consisted of support. There were no complications of treatment, but the disease eventuated in death in all instances (Table 1).

When Gilbert [2] proposed to treat Hodgkin's disease with radiotherapy, he made a major conceptual and therapeutic contribution which was first tested in a rigorous manner by Peters and Middlemiss in Toronto [3], and by Easson and Russell in Great Britain [4].

Because of their approach to the treatment of Hodgkin's disease with radiotherapy and because of their understanding of the limitations due to the technical aspects of such treatment, a new classification was devised and a staging technology proposed. This allowed Peters as well as Easson to identify stages I and II, which could be cured. Thus, Peters proposed three stages of Hodgkin's disease: involvement of a single site, stage I; involvement of 2 or 3 proximal lymphatic regions, with or without symptoms, stage II; and involvement of 2 or more distant lymphatic regions, stage III. The technological approaches to classifying patients consisted of a physical examination and radiological examinations comprising a chest film, intravenous pyelogram, and inferior vena cavagram. The aim of treatment was cure for stage I and II patients; the treatment modality consisted of orthovoltage radiotherapy given in wide fields. There were treatment complications, and of course, there were also treatment failures (Table 1).

The results, however, were astonishing compared to historical data. 5-year disease-free survival rates were 30%–40%, and 10-year disease-free survival rates were 20%–26% in Manchester and Toronto, respectively [3–5]. Peters' contribution was a landmark, since her selected cases had a 6-fold improvement in 5-year disease-free survival compared to the untreated cases reported by Croft in 1940 [6].

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