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Case report

Infusion of lymphocytes obtained from a donor immunised with the paraprotein idiotype as a treatment in a relapsed myeloma

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Summary:

A 48-year-old patient with IgA k multiple myeloma received a BMT from his HLA-matched sibling. After transplantation, the disease relapsed. Melphalan therapy followed by reinfusion of haemopoietic blood stem cells collected from the patient led to the improvement of the clinical status, although mixed chimerism and an elevated serum IgA persisted. Successful donor immunisation against an immunogenic preparation of the recipient monoclonal protein was performed before the infusion of donor T lymphocytes (DLI) into the patient. Ten weeks after the lymphocyte infusions, no monoclonal band was evidenced and donor complete chimerism was detected. The patient did not develop GVHD. Once complete remission was achieved, the idiotype vaccine was administered to the patient. Nineteen months after DLI, the patient remains in remission. *Bone Marrow Transplantation* (2000) **25,** 1105–1108.
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Intensive treatment with stem cell transplantation has been widely used in multiple myeloma (MM) patients in recent years.[1] Following allogeneic bone marrow transplant (BMT), there is a high transplant-related mortality, partially related to graft-versus-host disease (GVHD) and a high rate of relapse.

Treatment of relapse is not well established. The antitumour effect of donor lymphocyte infusions (DLI) is mediated by alloreactive T cells and is well documented in leukaemia. Moreover, incidental reports of remissions after DLI in small numbers of MM patients have indicated the existence of a graft-versus-myeloma (GVM) effect.[2–6] However, this technique has some disadvantages. On the one hand, the success of DLI has been limited to some extent by the morbidity and mortality associated with GVHD.[2,4,6] On the other hand, although a high response rate to DLI has been reported,[3,4] it is not always complete or durable, and long-term survival in complete remission is infrequent.[3,4]

An alternative strategy for treating relapsed MM patients after allogeneic BMT is to induce a specific immune response against tumour cells. Vaccine trials using malignant B cell idiotypes as tumour-specific antigens have yielded some promising results in patients with NHL.[7] In a similar way, the idiotype of the myeloma paraprotein can be used as a unique tumour-specific antigen. Kwak *et al*[8] immunised an allogeneic BMT donor against the recipient's idiotype protein and were able to transfer this donor immunity to the patient at the time of allografting. In a variation of this technique, immunisation of the donor before collecting cells for DLI could selectively boost GVM.

We present a case of relapsed MM after BMT in which complete remission was obtained after DLI from the histocompatible donor immunised with a myeloma idiotype (Id) vaccine.

Case report

A 48-year-old man who had been diagnosed as having an IgA kappa MM (IgA 7280 mg/dl, 36% plasma cells in bone marrow and vertebral compression fractures) had been treated at the age of 34 with allogeneic T cell-depleted (Campath I monoclonal antibody) BMT from an HLA-identical brother. The conditioning regimen included cyclophosphamide 120 mg/kg body weight and TBI 1200 cGy. Cyclosporin A was given for a year after transplantation. The post-BMT course was uneventful, without evidence of GVHD.

After transplantation, the paraprotein concentration remained around 800 mg/dl for a 6 year period. It then began to rise, having reached a level of 4510 mg/dl when we examined the patient at our hospital for the first time. At that time, the patient presented with generalised osteoporosis, vertebral compression fractures, lytic skeletal lesions, and 30% bone marrow plasma cells. PCR analysis of minisatellite sequences revealed mixed chimerism both in peripheral blood and bone marrow samples. The patient received three cycles of VAD chemotherapy, which improved the laboratory findings but not his clinical condition. Three months later, autologous peripheral blood

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haematopoietic stem cells were collected and re-infused after melphalan administration (140 mg/m^2). The patient showed clinical improvement and IgA decreased to 600 mg/dl (Figure 1).

Despite these features, the persistence of mixed chimerism and monoclonal paraprotein encouraged us to take a different approach in order to induce a more specific anti-tumour effect. Infusion of donor lymphocytes was planned and, in an attempt to increase the efficacy of the GVM effect, the donor was previously immunised against the recipient's monoclonal immunoglobulin. To this end, the IgA paraprotein was purified from an historical serum sample by MonoQ anion exchange chromatography plus Superose 12 gel filtration. The final product was pure as judged by agarose electrophoresis and SDS-PAGE. The purified IgA was coupled to keyhole limpet hemocyanin (KLH, Calbiochem, Darmstadt, Germany) with glutaraldehyde and dialysed against saline. Two doses of the idiotype vaccine (each containing 0.5 mg of IgA emulsified in SAF adjuvant) were administered to the donor 3 weeks apart.

The donor presented a self-limiting local inflammatory reaction at the site of the injection and a transient feeling of body heat loss after vaccination. In addition, a transient rise in serum anti-nuclear antibodies (ANA) to 1/160 was detected. There has been no evidence of any other clinical or analytical changes.

Anti-Id and anti-carrier (KLH) antibodies were detected in the donor serum by an enzyme-linked immunoassay (Figure 2, inset) 6 weeks after administration of the first dose. Seven weeks after the first idiotype vaccination, donor lymphocytes were collected and infused into the patient at a dose of 5×10^7 CD3$^+$ cells/kg body weight, while a second identical dose was cryopreserved and administered 6 weeks later. There was no evidence of GVHD during this period.

Eight weeks after the first infusion, the serum IgA concentration had decreased to 211 mg/dl (Figure 1) and a faint band of monoclonal IgA was disclosed by immunofixation; the peripheral blood chimerism was complete for the first time. Ten weeks later, the IgA concentration was 102 mg/dl and immunofixation revealed no evidence of monoclonality. On this date, the bone marrow contained 2% plasma cells and serum IgG and IgM reached normal values.

The only noteworthy incident occurred 8 weeks after the first infusion, when the patient presented signs of small joint inflammation. He was successfully treated with non-steroid anti-inflammatory drugs. Rheumatoid factor and ANA levels, which had risen to 176 IU/ml and 1/320 during the first weeks after DLI, are currently $<$20 IU/ml and 1/80, respectively. Transaminase levels rose moderately during the first 3 months after DLI, and began to decrease thereafter to normal levels.

Anti-Id and anti-KLH antibodies were not detected in the recipient serum during a 6-month follow-up period after DLI (Figure 2). In this case, the presence of low amounts of paraprotein, undetectable by immunofixation, that could absorb the auto-anti-idiotypic antibodies was excluded because the addition of patient serum did not inhibit the reactivity of the anti-idiotypic antibodies present in donor serum (data not shown).

Once complete remission was achieved, two doses of idiotype vaccine (at a 5-week interval) were administered to the patient for the purpose of enhancing and perpetuating the anti-tumour effect. A positive response (anti-Id and anti-KLH) was evident 1 month after the last immunogen injection (Figure 2). There has been no evidence of any clinical or analytical disturbances after the idiotype vaccination. Nineteen months after the first DLI, the patient remains in complete remission.

Discussion

Even though allogeneic haematopoietic stem cell transplant is the only curative treatment for MM, the highly variable results obtained are an impetus to search for new therapeutic approaches. The relapse of MM in our patient was

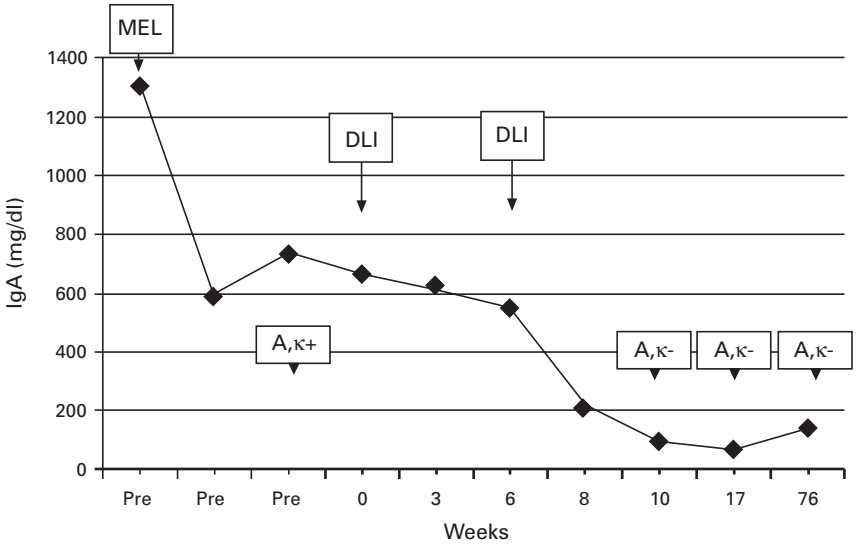

Figure 1 Serum IgA concentrations in the patient's serum. Melphalan (MEL) and donor lymphocyte infusions (DLI) were given at the indicated times (closed arrows). Immunofixation initially revealed a monoclonal IgA component (A,κ+), but disclosed no monoclonality after treatment (A,$\kappa-$).

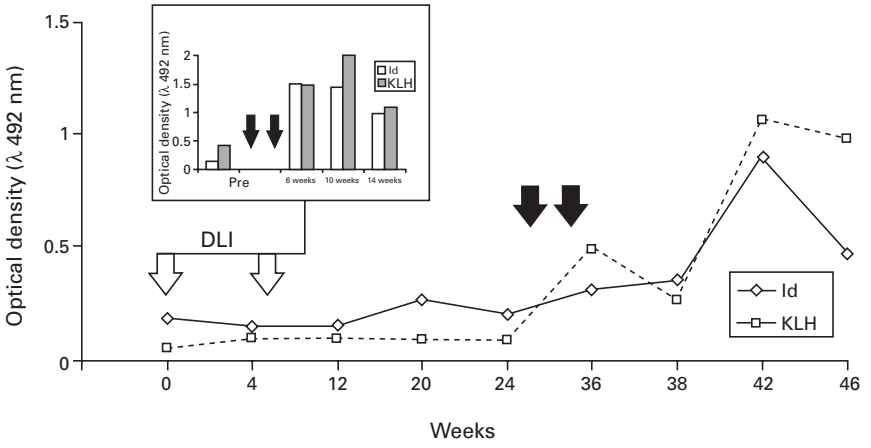

Figure 2 Anti-idiotypic and anti-KLH antibodies in the sera of donor (inset) and recipient of DLI. Donor lymphocytes were obtained 7 weeks after the first immunisation with an Id-KLH conjugate (inset, closed arrows) and infused into the patient (open arrows). Two doses of Id-KLH were given at weeks 28 and 33 (closed arrows). ELISA results at optimal dilutions (1:8 for anti-Id, 1:1000 for anti-KLH antibodies) are shown.

first treated with high-dose chemotherapy followed by administration of haematopoietic stem cell progenitors previously obtained from the patient's blood. Although the tumour burden was reduced, the persistence of serum paraprotein and mixed chimerism led us to look for a more effective therapeutic approach.

A GVM effect has been documented in a few myeloma patients who received leukocyte transfusion from their initial donor after having relapsed despite allogeneic BMT.[2–6,9] The optimal T cell doses for inducing remission in MM have not been definitively established,[5] but there is an association between higher T cell dose and response.[4,5] This is probably due to a close relationship between GVHD and GVM, as the incidence of GVHD appears to be higher in patients who receive larger numbers of donor cells.[3,4] In fact, it has been suggested that, as GVHD appears to be at least a surrogate marker for a subsequent GVM effect, the correct dose of T cells would appear to be that which causes some GVHD.[5] Nevertheless, the success of DLI has been limited to some extent by the morbidity and mortality associated with GVHD.[2,5,10] To avoid this complication, and in an attempt to induce a specific immune response against myeloma cells, we decided to infuse a lower number of donor T cells after immunising the donor against the patient's idiotype protein.

The present report is the first describing a case of MM relapsed after BMT, treated with lymphocyte infusions from a donor immunised with an idiotype vaccine prepared from purified tumour paraprotein. A complete response was obtained, as was demonstrated by a normal bone marrow biopsy, complete chimerism and the disappearance of the paraprotein. These findings may indicate that the specific immunological response induced by idiotype vaccination in the donor is transferred to the patient after the administration of the allogeneic blood lymphocytes. Although it could be argued that the patient may have responded to DLI rather than to donor immunisation, several reasons make it unlikely that the anti-tumour effect in this patient resulted from DLI alone.

First, lymphocytes were collected from the donor once anti-idiotype and anti-KLH antibodies were detected in his serum, which proves the success of donor immunisation just before lymphocyte collection. Therefore it can be supposed that donor T cells may have a selective action against myeloma cells. Second, although the optimal T cell doses for inducing remission in MM are not definitively established and variable cell doses have been used to elicit GVM, the best response rates have been associated with infusion of $>1 \times 10^8$ T cells/kg body weight.[3–5] Lokhorst *et al*[4] found that a T cell dose of more than 1×10^8 cells/kg induced a response in four of six patients (67%), compared with only four responses after 21 DLI containing 1×10^8 T cells/kg or less (19%). It generally takes 4–10 weeks for a response to become evident after DLI, and at least a few months to attain complete remission.[3–5] Our patient received two doses of 5×10^7 T cells/kg, 6 weeks apart. Given the short time which elapsed between the second DLI and the first signs of a response, we think that the results are more likely due to the effect of the first lymphocyte infusion than to the total dose infused (1×10^8/kg). On the other hand, patients who responded to lower doses have been reported, but they usually developed GVHD,[2,5] which was absent in our patient. Third, there is a strong correlation between GVHD and GVM, and the development of GVHD is highly associated with response.[3–6] Nevertheless, responses can occur in the absence of GVHD,[3] but they are often partial responses, and most MM in patients without evidence of GVHD eventually progress.[3,4] Moreover, in some patients the disease remains in remission while there is active GVHD and relapse often occurs as the GVHD comes under control.[10] In contrast, our patient showed no evidence of GVHD, and not only achieved a complete response, but remains in remission 19 months after DLI.

In conclusion, in the context of relapsed myeloma after allogeneic haematopoietic BMT, idiotype vaccination of the donor may enhance the GVM effect of DLI, helping to induce a complete remission. Although it deserves further study, this strategy may permit the infusion of lower doses of DLI to control the disease, reducing the probability of inducing GVHD.

Once complete remission is achieved, an idiotype vac-

cine could be administered to the patient in order to enhance and perpetuate the graft-versus-tumour effect. In our case, a positive anti-Id and anti-KLH response was observed in the patient serum 1 month after the last vaccine dose, a finding that may be of importance in terms of the future outcome of the treatment.

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