

## PREVENTION OF BONE MARROW GRAFT FAILURE BY AN ANTI LFA-1 MONOCLONAL ANTIBODY

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*Graft rejection is the major cause of failure of HLA mismatched bone marrow transplantation because of residual host immunity. We have proposed to use a monoclonal murine antibody specific for the LFA-1 molecule (25-3) to prevent graft failure in HLA mismatched bone marrow transplantation (BMT). The rationale for this approach is three fold : LFA-1 deficient patients (3/3) do not reject HLA mismatched BMT; anti LFA-1 blocks in vitro the induction of T cell responses and T/non T cytotoxic functions; LFA-1 is not expressed by other cells than leucocytes. We have accordingly treated twenty two patients with inherited diseases and 8 with leukemia. The bone marrow was T cells depleted by E rosetting of Campath antibody. The antibody was given at days -3, -1, +1, +3, +5 at dose of .1mg/kg/d for the first 9 and then .2mg/kg/d from day -3 to +6. Engraftment occurred in 23/30 patients as shown by at least HLA typing. Hematological recovery was rapid, GVHD was limited. Side effects of antibody infusion included fever and possibly an increased incidence of early bacterial infections (sepsis, 1 death). Immunological reconstitution occurred slowly leading in six cases to EBV-induced B cell proliferation (1 death) and in two others to transient auto immune hemolytic anemia. There has been only one secondary graft rejection. Sixteen patients are alive 3 to 26 months post transplant with functional grafts. Although the number of patients treated is still low the absence of late rejection so far, gives hope for long term maintenance of the graft using anti LFA-1 antibody. Since the antibody is an IgG1 unable to bind human complement, and since it is known to inhibit phagocytosis, there is a good suggestion that 25-3 acts through functional blocking of host T and non T lymphocytes at both induction and effector levels.*

Graft rejection is the major cause of failure of HLA mismatched bone marrow transplantation (BMT) (O'Reilly et al., 1984). The incidence of graft rejection following T cell depleted HLA mismatched transplant ranges between 40 and 90%. The risk is lower if donor T cells are not removed from the marrow inoculum. However, in this instance there is an extremely high incidence of graft versus host disease (GVHD) (over 90%) (Beatty et al., 1986) which causes lethality. Graft rejection is mediated by residual host immunity. Indeed, patients with severe combined immunodeficiency are unable to reject mismatched bone marrow transplants (Fischer et al., 1986a, b). Reisner et al. (1986) have shown that cytotoxic T cells can resist lethal total body irradiation in the mouse and the monkey. T8<sup>+</sup> lymphocytes have been demonstrated to be responsible for graft destruction in the piglet model. In man, activated cytotoxic T lymphocytes have been found in the blood at time of graft rejection. It is also possible that NK-like cells are involved through antibody dependent cellular cytotoxicity (Warner & Dennert, 1985).

Several approaches are currently used to overcome graft rejection. In animal models additional immunosuppressive drugs and increased dosage of total body irradiation and/or total lymphoid irradiation are efficient means to abrogate residual host T/non T cells. However such procedure is not amenable to men because of unacceptable toxicity. More selective immunosuppression can be delivered through the infusion of monoclonal antibodies. In the mouse, Cobbold et al. (1986) have shown that the in vivo infusion of anti T4 and T8 antibodies to mouse have allowed the acceptance of H2 fully incompatible bone marrow. In the dog, an anti Ia antibody has also been efficiently used.

We have chosen to use an anti LFA-1 antibody to prevent bone marrow graft failure in men for the following reasons.

There is a rare inherited immunodeficiency, characterized by a selective absence of three adhesion proteins called LFA-1, CR 3 and p150.95 which are heterodimers selectively expressed by leukocytes (Springer et al., 1984).

The deficiency is due to an abnormal synthesis of the B chain common to the 3 molecules. The disease is above all a deficiency of phagocytic cell functions since the latter cells are unable to adhere to endothelial cells and eventually to migrate to infections sites. The CR3 and p150.95 molecules electively expressed by phagocytic cells are responsible for these functions. Cytotoxic functions of T and non T lymphocytes are also impaired (Fischer et al., 1985). Interestingly, in contrast with the frequency of graft failure (7/8) observed after HLA mismatched marrow transplants performed for other partial immunodeficiencies, in 3/3 occasions a stable engraftment of HLA incompatible marrows occured in this disease (Fischer et al., 1986b). We have thus postulated that LFA-1(-) lymphocytes are unable to reject an incompatible bone marrow because cytotoxic lymphocytes fail to adhere to their targets. We therefore reasoned that by reproducing such a deficiency, HLA incompatible graft could be accepted. In order to block LFA-1 dependent functions we are using a mouse monoclonal antibody "25.3" of IgG1 isotype specific for the alfa chain of LFA-1 (D. Olive et al., 1986). This antibody has been shown in vitro to strongly suppress cytotoxic T and non T functions as well as to partially inhibit mixed leukocyte reaction. Further argument for the use of such an antibody has been brought up by Heagy et al. (1984) who gave evidence for an anti LFA-1 antibody to be most potent immunosuppressive antibody as judged on the prevention of the rejection of an allogeneic tumor in the mouse.

Finally, the LFA-1 molecule is not expressed on other tissues and anti LFA-1 antibodies do not inhibit the growth of hematopoietic progenitor cells (CFU-GM, CFU-Eo, BFU-E, CFU GEMM) indicating that the molecule is not expressed by stem cells (Campana et al., 1986). There was thus no indication of a possible hazard in the in vivo infusion of the anti LFA-1 antibody.

#### PATIENTS AND METHODS

We first considered patients with potentially lethal diseases who had a partial immunodeficiency. A control historical group of such patients treated with a partially HLA incompatible, T cell depleted bone marrow gave poor results. Among 7 patients, a stable, although partial, engraftment occured in only one. In all others there was a primary failure of engraftment. None of the patients survived with a

functional graft. From June 1985 until June 1987 thirteen patients with partial immunodeficiency have received a partially HLA incompatible bone marrow using the same protocol as for the historical control group except that, in addition, they received anti LFA-1 antibody infusion. Diseases included Wiskott-Aldrich syndrome (9) and combined immunodeficiencies (4). More recently patients with various inherited disorders without immunodeficiencies were also considered for similar BMT because of their poor natural prognosis. They include 9 patients (7 with osteopetrosis, one with Gaucher's disease and one with metachromatic leucodystrophy). Another 6 patients with high risk leukemia in remission or in chronic phase have also been likewise transplanted as well as 2 patients in acute phase of juvenile chronic myelomonocytic leukemia.

The degree of HLA incompatibility between donor (usually parents) and recipient was the following : in one case 1 HLA DR incompatibility, 2 antigens in 9 and one haplotype in 20.

We first used the anti LFA-1 antibody according to the following regimen.

.1 mg/kg at days -3, -1, +1, +3 and +5 around the date of transplantation. However the antibody in the serum measured 24 hr following a 4 hr infusion was undetectable as measured by an Elisa assay. We therefore decided to give .2 mg/kg daily from day -3 to +6. Using this protocol, the serum antibody concentration reached a plateau around 1  $\mu$ g/ml for several days.

#### RESULTS AND COMMENTS

In most of the cases, the infusion of the anti LFA-1 antibody was well tolerated. In a few instances, it induced transient fever.

The overall results are depicted in the Table. The rate of engraftment is good. Most of the failures but one have been primary failures of engraftment. Three out of 7 failures occured in patients with osteopetrosis a condition known to be associated with poor engraftment of HLA matched bone marrow because of the absence of medullary space. The hematological recovery was in average not different to that observed after HLA matched BMT. Graft versus host disease was not a major problem in most of the cases although two patients have died from severe acute GVHD and one has a persistent but limited chronic GVHD.

TABLE  
Overall results

	No.	Engraftment	Alive with functional graft (follow up in month, mean, range)
<b>Immunodeficiencies</b>			
• Control group	7	1	0
• anti LFA-1 group	13	11	9 (10, 3-26)
<b>Metabolic diseases</b>			
• Control group	3	0	0
• anti LFA-1 group	9	6	4 (10, 3-26)
<b>Leukemia</b>			
• in remission or chronic phase	6	5	3 (5, 3-6)
• in relapse	2	1	0
<b>Total</b>			
• Control group	10	1	0
• anti LFA-1 group	30	23	16 (9, 3-26)

The major complications encountered in these patients were infections that can be divided in to two groups:

a) early bacterial infections within the first month. Six cases of sepsis, one being fatal have been observed among the first twelve patients. This high incidence is probably secondary to an inhibitory effect of the anti LFA-1 antibody on the ability of tissue macrophages to clear bacterias and fungi. The prophylactic infusion of antibiotics has thereafter controlled this risk.

b) late viral infections. Viral infections such as cytomegalovirus induced pneumonitis or Epstein-Barr virus induced B lymphocyte proliferative syndrome have been observed in 2 and 6 cases respectively, causing death in 3 occasions. There is clearly a prolonged T and B immunodeficiency following such HLA incompatible BMT that underlies the risk of severe and unusual viral infections. The reason for the delay in T and B cell functions developement (6 and 6 to 12 months respectively) remains unknown, but is unlikely due to the infusion of the anti LFA-1 antibody which has a short life. This immunodeficiency requires greatest care of the patients within the first six months following BMT.

The results undoubtedly indicate that use of the anti LFA-1 antibody contributes to the engraftment of HLA incompatible bone marrow. Immediate adverse effects are neglectable although late infections remain a major problem. The mechanism by which the anti LFA-1 antibody acts is not entirely clear.

Since the antibody is an IgG1, complement dependent cytotoxicity does not play any role. The apparent most obvious mechanism could have been opsonization and phagocytosis of LFA-1(+) cells. However the antibody has a strong blocking effect on phagocytosis and macrophages do express LFA-1. One is thus left with the possibility that the anti LFA-1 antibody acts through a functional blocking effect on the lymphocytic effector cells. Indeed anti LFA-1 antibodies can block in vitro to some extent the induction of T cell activation as well as the effector functions especially cytotoxicity. Confirmation of this mechanism would need experimental models.

An other field of speculation deals with the persistence of an effect mediated by a short course of antibody infusion (10 days). It may well be that the antibody covers a narrow window within the first month following BMT during which donor T cells have not yet developed, allowing thus potential rejections. It is indeed known that donor mature T cells can prevent graft rejection, the mechanism remaining unexplained.

Much efforts are still needed in order to determine the optimal regimen of infusion, to delineate the indications and to know for instance whether such antibody could be used for other immunosuppressive purpose.

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