Efficiency of Antibodies Directed Against Adhesion Molecules to Prolong Skin Graft Survival in Mice

Y. van Kooyk, A. de Vries-van der Zwan, L.P. de Waal, and C.G. Figdor

THE interest to use monoclonal antibodies (MAbs) as immunosuppressive agents in allograft rejection treatment has increased considerably. Certain MAbs can block or modify critical steps in the rejection response and have the capacity to prolong graft survival, without the appearance of toxic side effects. We have studied the potential capacity of anti-adhesion molecules MAbs to inhibit the rejection of skin grafts in mice. This study focuses on the adhesion molecules that are expressed on leukocytes and mediate distinct adhesion interactions between leukocytes and between leukocytes and endothelium. LFA-1 (CD11a/CD18) is such a leukocyte-specific adhesion molecule, which binds its ligand ICAM-1 (CD54), present on leukocytes as well as endothelium.[1,2] By using inhibitory MAbs directed against these adhesion receptors, in vitro studies have demonstrated that this receptor-ligand interaction (LFA-1–ICAM-1) is required for antigen-presenting cell (APC)–T-cell interaction, T–B-cell interaction, T-cell–mediated killing, as well as T-cell migration.[3] Recently also other ligands of LFA-1 have been identified (ICAM-2 and ICAM-3), which may also contribute in these distinct LFA-1-mediated cell-cell interactions.[4] Apart from LFA-1, another adhesion receptor (Mac-1) has been described to bind ICAM-1.[5] Mac-1 (CD11b/CD18) is a member of the LFA-1 family (CD18 family) expressed by monocytes and granulocytes and is involved in inflammatory responses mediated by these cells. Apart from ICAM-1 other ligands are recognized by Mac-1.[6]

Here we investigated if MAbs directed against LFA-1, Mac-1, ICAM-1, or a combination of these antibodies could enhance skin graft survival in mice.

MATERIALS AND METHODS

Animals

Each group consisted of five male C57BL/6 (H-2^b) (B6) recipient mice that were transplanted each with two male bm1 (H-$2D^bK^{bm1}$) tail skin grafts and one syngeneic skin graft on the upper part of the tail. One day before transplantation (day −1) mice were injected intraperitoneally with 300 μg antibody or a combination of 300 μg of each antibody, followed by injections twice a week, until the end of the experiment (maximal 80 days).

Antibodies

The rat MAbs M17.4 obtained from the American Type Culture Collection (ATCC, Rockville, Md) and H154.163, kindly provided by Dr Pierres,[7] were directed against the murine LFA-1 α-chain; MAb YN1/1.7.4, obtained from ATCC, was directed against murine ICAM-1. The MAbs M1/70.15, obtained from ATCC, and 5C6, kindly provided by Dr M. Robinson, were directed against the murine Mac-1 α-chain.

Cell-mediated lympholysis

CML was performed by culturing B6 spleen cells for 5 days at 37°C with allogeneic bm1 irradiated spleen cells in Iscove's medium supplemented with 5% fetal calf serum (FCS). The cytotoxic response of the effector cells was tested by their capacity to kill ^{51}Cr-labeled bm1 lipopolysaccharide blasts in a ^{51}Cr release assay of 4 hours at 37°C. Antibodies were added during the effector phase and were used in a final concentration of 10 μg/mL.

RESULTS

The blocking capacity of different anti-adhesion MAbs in adhesion-dependent immunologic processes, such as effector-target cell interaction, was investigated in vitro. The capacity of two anti-LFA-1 (M17.4, H154.163), two anti-Mac-1 (M1/70.15, 5C6), and one anti-ICAM-1 (YN1/1) MAb to block the cytotoxic B6 anti-bm1 response was determined. Both anti-LFA-1 antibodies inhibited the specific lysis of bm1 target cells by the B6 effector cells, anti-ICAM-1 antibodies inhibited partially, whereas anti-Mac-1 did not (Fig 1). The combination of anti-LFA-1 and ICAM-1 antibodies completely inhibited cytolysis. All antibodies that blocked the cytotoxic T-lymphocyte (CTL) response were also potent inhibitors of the B6 anti-bm1 MLR (mixed lymphocyte reaction, not shown). Although Mac-1 antibodies did not inhibit the cytotoxic or proliferative response of the allogeneic bm1-induced B6 response, these antibodies inhibited other Mac-1–dependent interactions (not shown).

The in vivo capacity of these antibodies to inhibit skin transplant rejection was determined by injecting mice intraperitoneally with 300 μg of each antibody, starting 1 day before transplantation, followed by injections twice a week until the end of experiment. This protocol was chosen because 300 μg yielded high serum levels and occupied the adhesion receptors expressed on lympho-

From the Division of Immunology, The Netherlands Cancer Institute (Y.K., C.G.F.), Antoni van Leeuwenhoek Huis, and Central Laboratory Blood Transfusion Service (A.V., L.P.W.), Amsterdam, The Netherlands.

Supported by the Dutch Kidney Foundation ("de Nierstichting") grant C87-724.

Address reprint requests to Dr Yvette van Kooyk, Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

0041-1345/94/$3.00/+0

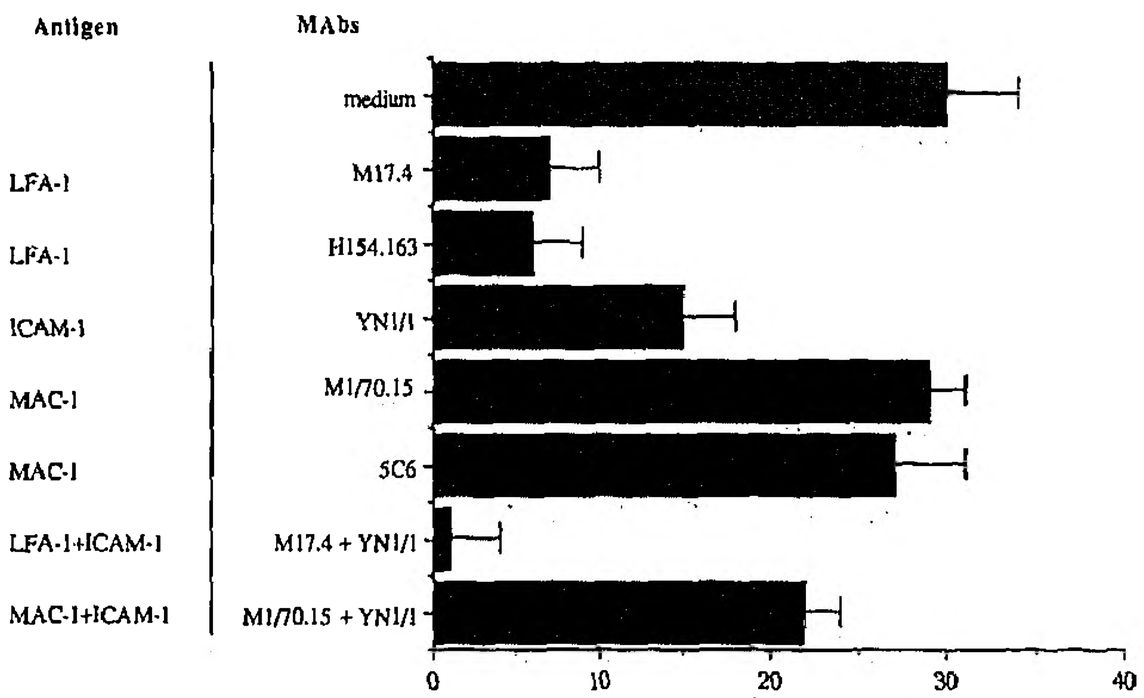

Fig 1. Inhibition of the B6 anti-bm1 cytolytic response by antibodies directed against adhesion molecules during target cell lysis.

cytes, present in spleen or lymph nodes, for more than 3 days. No side effects were observed after injection of the mice twice a week with any of the MAb used for a period of more than 10 weeks. Each B6 mouse was transplanted with two allogeneic bm1 skins and one syngeneic B6 skin. Syngeneic skins were not rejected during the experiment. In contrast, mice rejected the allogeneic bm1 skins within 24 days. Treatment of recipient mice with anti-ICAM-1 antibodies (YN1/1) did not prolong allograft survival, skins were rejected within 30 days (Table 1). Treatment of the recipient mice with the anti-Mac-1 antibodies (5C6 or Mac-1) enhanced graft survival, however transplants were still rejected between days 35 to 40. In contrast, when recipient mice were treated with anti-LFA-1 antibodies (M17.4 or H154.163), acceptance of the allogeneic graft was observed for more than 80 days. Both anti-LFA-1 antibodies (M17.4 or H154.163) improved graft acceptance, although mice injected with H154.163 showed a more stable transplant survival (small SD) compared with mice injected with M17.4 (large SD). Recipient mice treated with a combination of anti-LFA-1 and anti-ICAM-1 antibodies also showed a prolonged survival of the transplant similar to mice injected with anti-LFA-1 MAb alone. When mice were treated with a combination of anti-Mac-1 and anti-ICAM-1 MAbs no prolongation of the skin survival was observed, indicating that the partial transplant acceptance obtained by anti-Mac-1 alone is inhibited when anti-ICAM-1 antibodies are added.

DISCUSSION

We have shown that treatment of mice with M17.4 or H154.163 (anti-CD11a) potently and effectively prolong survival of bm1 skin grafts in B6 mice for more than 80 days. This indicates that LFA-1 plays a central role in transplant rejection by mediating several cell-cell interactions. Moreover, the combination of anti-LFA-1 and anti-ICAM-1 antibodies did not show any additional effect on the survival time of the transplant when compared with anti-LFA-1 alone. In contrast to the effect observed with anti-LFA-1 MAb alone, no survival of the grafts was found when mice were treated with YN1/1 (anti-ICAM-1). Thus, the finding that anti-LFA-1 blocks graft rejection whereas anti-ICAM-1 MAb does not, suggests that inhibition of other ligands of LFA-1, that is, ICAM-2 or ICAM-3 may be more important than LFA-1–ICAM-1 for survival of the transplant. On the other hand other anti-ICAM-1 antibodies may be more effective than the one used in this study.

The fact that anti-ICAM-1 MAbs were not potent in prolonging graft survival could also be explained by the fact that anti-rat antibodies were found in serum after injecting mice with anti-ICAM-1. In contrast no anti-rat antibodies were observed in mice that were injected with anti-LFA-1 antibodies alone, because anti-LFA-1 blocked the antibody response by B cells. The mechanism by which immunosuppression is achieved through anti-LFA-1 is not known. LFA-1 plays an important role in the homing of lymphocytes through binding ICAM-1, or -2, thus an altered cell migration may be one mechanism by which allograft survival is prolonged. On the other hand the LFA-1–ICAM-3 interaction has been suggested to play an essential role in the induction of an immune response, and thus may be an important step to induce transplant rejection.

Our findings are in line with the recent observation of Nakakura et al[8] who demonstrated that the same anti-LFA-1 antibody (M17.4) results in a prolonged heart allograft survival in mice. We have also observed that no tolerance was induced in the treated mice, allogeneic skins

Table 1. Survival in Days of Skin Allografts (H-2D^{b}K^{bm1}) Transplanted on B6 (C57Bl/6; H-2D^{b}K^b) Mice

Antigen	MAbs	No.	Survival d	Mean Survival d ± SD
—	None	5	22, 22, 24, 26, 28	24.4 ± 2.6
ICAM-1	YN1/1	5	22, 24, 24, 28, 50	29.6 ± 11.6
MAC-1	M1/70.15	5	26, 30, 42, 42, 62	40.4 ± 14.0
MAC-1	5C6	5	26, 32, 35, 38, 45	35.2 ± 7.0
LFA-1	M17.4	5	24, 30, 66, 72, 192	76.8 ± 67
LFA-1	H154.163	5	44, 80, 80, 98, 98	80.0 ± 22
LFA-1 + ICAM-1	M17.4 + YN1/1	5	20, 28, 48, 99, 184	75.8 ± 67
MAC-1 + ICAM-1	M1/70.15 + YN1/1	5	16, 22, 22, 24, 54	27.6 ± 11.6

Note. Recipient mice were injected with 300 μg of each MAb starting 1 d before transplantation, followed by twice a week until the end of experiment (maximal 80 d).

where ultimately all rejected. This is in contrast to the experiments described by Isobe et al (9) who have demonstrated that tolerance was induced in mice by a combination of anti-LFA-1 and anti-ICAM-1 when transplanted with allogeneic hearts.

Although anti-Mac-1 antibodies (Mac-1 and 5C6) did not inhibit in vitro responses, when injected in vivo a significant prolongation of graft survival was observed. However, a much longer graft survival rate was observed with anti-LFA-1. This indicates that Mac-1–dependent adhesion of monocytes and granulocytes plays a less important role in the process of graft rejection than LFA-1. Because a humoral response was detected in mice injected with anti-Mac-1 antibodies, the formation of anti-rat antibodies could also attribute to the minor affects seen with anti-Mac-1 antibodies.

In conclusion we have demonstrated that antibodies directed against the adhesion molecules LFA-1, and to a lesser extent, Mac-1 lead to prolonged skin graft survival in mice.

ACKNOWLEDGMENTS

We thank Dr M. Robinson and Dr M. Pierres for kindly providing the antibodies 5C6 and H154.163, respectively.

REFERENCES

1. Martin SD, Springer TA: Cell 51:813, 1987
2. Springer TA: Nature 346:425, 1990
3. Krensky A, Sanchez-Madrid F, Robbins E, et al: J Immunol 131:611, 1983
4. de Fougerolles AR, Stacker SA, Swarting R, et al: J Exp Med 174:253, 1991
5. Diamond MS, Staunton DE, de Fougerolles AR, et al: J Cell Biol 111:3129, 1990
6. Davids DE: Exp Cell Res 200:242, 1992
7. Pont S, Naquet P, Marchetto S, et al: J Immunol 136:3750, 1986
8. Nakakura EK, McCabe SM, Zheng B, et al: Transplant Proc 25:809, 1993
9. Isobe M, Yagita H, Okumura K, et al: Science 255:1125, 1992