22. We obtained the apparent kinetic parameters used in these calculations as described (*3–6*), taking into account the technical issues associated with the differential quenching of ^{3}H-labeled amino acids (*19*). We obtained the micro^{Ala} and micro^{His} kinetic parameters by measuring the initial rates of aminoacylation of microhelices over a greater concentration range than that used in previous work (1 to 500 μM versus 1 to 50 μM [RNA]), and this gives a lower apparent V_{max}/K_m for micro^{Ala} (Table 1) than reported (*3*). The k_{cat} parameter may vary with the degree of saturation of the tetrameric AlaRS.
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Specific Acceptance of Cardiac Allograft After Treatment with Antibodies to ICAM-1 and LFA-1

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An indefinite survival of cardiac allografts between fully incompatible mice strains was observed when monoclonal antibodies (MAbs) to intercellular adhesion molecule–1 (ICAM-1) and leukocyte function–associated antigen-1 (LFA-1) were simultaneously administered after the transplantation for 6 days. Mice with long-term surviving cardiac allografts accepted skin grafts from the donor-strain but rejected skin grafts from a third-party strain. Because MAbs to ICAM-1 or LFA-1 alone were insufficient for prolonged tolerance, the two MAbs probably acted synergistically to induce specific unresponsiveness. Thus, ICAM-1–LFA-1 adhesion participates in the induction of allograft rejection and MAbs may be useful as therapeutic agents.

ADHESION MOLECULES PARTICIPATE in the many stages of an immune response (*1, 2*). T cell immune recognition requires the contribution of the T cell receptor as well as adhesion receptors, which promote attachment of T cells to antigen-presenting cells (APCs) and transduce regulatory signals for T cell activation. The LFA-1 and ICAM-1 adhesion molecules form one such heterophilic receptor-ligand pair (*3, 4*). LFA-1 is required for a range of leukocyte functions, including lymphokine production of helper T cells in response to APCs, killer T cell–mediated target cell lysis, and immunoglobulin (Ig) production through T cell–B cell interactions (*2, 5*). Activation of antigen receptors on T cells (*2*) and B cells (*6*) allows LFA-1 to bind its ligand with higher affinity. Also, the LFA-1–ICAM-1 interaction is required for optimizing T cell function in vitro (*7, 8*). Therefore, MAbs to these molecules are potential agents for the prevention of graft rejection (*9*). In this report, we demonstrate the effect of the combination of these two MAbs on allograft survival, which leads to specific tolerance in a mouse heterotopic cardiac allograft model.

The MAbs used in this study, KBA (IgG2a) (*10*), M18/2 (IgG2a) (*11*), and YN1/1.7 (IgG2b) (*12, 13*) are rat MAb to mouse CD11a (α chain of LFA-1), CD18 (β chain of LFA-1), and ICAM-1, respectively (*14*). Because M18/2 does not block cell-mediated target cell lysis in vitro (nonneutralizing MAb) (*11*), we used M18/2 as control MAb. Hybridoma cells that produce these MAbs were cultured in RPMI 1640 supplemented with 10% fetal bovine serum and 0.1% gentamycin. The MAbs from ascites produced in nude mice were purified with the use of a protein G affinity column.

BALB/c (H-2^d) hearts were heterotopically transplanted into C3H/He (H-2^k) recipients by a microsurgery technique (*15, 16*). Survival of cardiac grafts was assessed by daily palpation, and the cessation of graft beat was interpreted as the completion of rejection (*16*). Because of the full incompatibility of the H-2 complex, control C3H/He mice without any treatment invariably rejected BALB/c heart allografts within 10 days (Table 1). Treatment of recipient mice with M18/2 did not prolong allograft survival. Animals treated with daily doses of 100 μg of either YN1/1.7 or KBA showed significant prolongation of allograft survival when compared to the control or M18/2-treated mice. However, all these animals rejected allografts within 50 days. In contrast, all nine animals treated with 50 μg of YN1/1.7 together with 50 μg of KBA accepted cardiac allografts as long as the observation was continued (75 to 200 days). The intensity and frequency of beating of these allografts were indistinguishable from those of isografts.

Histological examination of the grafts treated with the two MAbs showed greatly reduced mononuclear cell infiltration at the seventh day as compared with control allografts without treatment (Fig. 1, A and B). The treated allografts removed at 40, 75 (Fig. 1C), and 120 days after operation showed only scattered areas of fibrosis without any evidence of active rejection.

Cytotoxic T lymphocyte (CTL) activity of

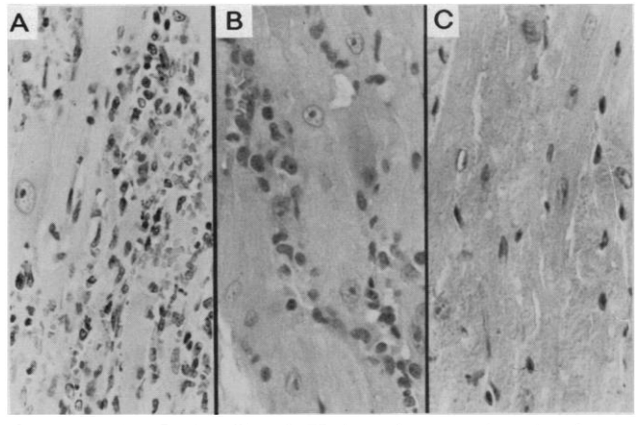

Fig. 1. Histological analysis of BALB/c heart allografted in C3H/He recipient. (**A**) A control allograft 7 days after the transplantation with no immunosuppressive treatment. Massive infiltration of leukocytes together with myocyte necrosis and interstitial hemorrhage are noted. (**B**) An allograft 7 days after transplantation from a mouse that was treated with a 6-day course of YN1/1.7 and KBA MAb commencing immediately after the transplantation. Diffuse interstitial infiltrates are noted, but the myocytes are free from necrosis. (**C**) An allograft 75 days after transplantation from a recipient that was treated as in (B). No sign of active rejection is seen. All sections are fixed in 10% formalin and stained with hematoxylin-eosin. Original magnification is ×200.

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splenocytes from the recipients was tested on day 7, 40, and 75 after transplantation (Table 2). On day 7 splenocytes from allografted recipient mice without MAb treatment showed higher CTL activity against donor H-2-compatible target cells (P815, H-2^d) than splenocytes from normal ungrafted mice. The spleen cells from allografted mice treated with KBA or treated with both KBA and YN1/1.7 did not show any increase in CTL activity. The treatment with YN1/1.7 alone resulted in only weak inhibition of the CTL induction. No enhanced CTL activity was observed 40 or 75 days after transplantation in the recipients that received both KBA and YN1/1.7.

To further evaluate the tolerant state of these mice, we challenged them with skin grafts. Five mice that apparently accepted the cardiac allografts were transplanted with donor-syngeneic (BALB/c) and third-party (C57BL/6, H-2^b) body skin simultaneously. All animals normally rejected third-party skin, but they accepted donor-syngeneic skin (Fig. 2). All cardiac grafts continued to beat during this observation period. Thus, allospecific tolerance was established in these mice.

The mechanism of this sustained unresponsiveness has yet to be established. As cell adhesion mediated by the LFA-1–ICAM-1 system is an essential part of various T cell functions in vitro, they are involved in eliciting primary immune response to alloantigens in vivo. Costimulatory signals mediated by some accessory molecules are required for T cell activation and clonal expansion. T cell receptor–mediated stimulation in the absence of a costimulatory signal can lead to T cell inactivation (*17*). LFA-1 is a candidate molecule responsible for transmitting this costimulatory signal upon binding to its ligand, ICAM-1 (*3, 8*). Therefore, the continuous stimulation with alloantigens in the absence of the LFA-1–mediated costimulatory signal by MAb blocking LFA-1/ICAM-1 interaction might lead to the induction of this specific tolerance.

A 6-day course of antibody to ICAM-1 (anti–ICAM-1) and antibody to LFA-1 (anti–LFA-1) treatment of ungrafted C3H/He mice did not reduce circulating leukocyte count [treated, 6.5 ± 0.6 × 10^3 per cubic millimeter (mean ± SD) versus normal, 6.1 ± 1.0, both $n = 3$] or yield of leukocytes per

Table 1. Survival days of cardiac allografts (BALB/c) transplanted into C3H/He mice. Recipient mice were injected daily with either 100 μg of M18/2, YN1/1.7, or KBA alone or 50 μg of YN1/1.7 plus 50 μg of KBA starting immediately after surgery and lasting until the sixth day of transplantation. Survival time in mice treated with YN1/1.7 and KBA was significantly greater ($P < 0.05$) than that in untreated, M18/2-treated, YN1/1.7-treated, or KBA-treated mice.

Treatment	*n*	Survival days	Mean survival (days ± SD)
None	6	7, 7, 8, 8, 8, 10	8.0 ± 1.1
M18/2	6	7, 8, 8, 9, 9, 10	8.8 ± 1.2
YN1/1.7	6	11, 12, 12, 13, 15, 23	14.3 ± 4.5
KBA	6	17, 20, 25, 30, 38, 47	29.5 ± 11.3
YN1/1.7 plus KBA	9	>70, >70, >70, >70, >70, >70, >70, >70, >70	>70

Table 2. Cytotoxic T lymphocyte assay. Recipient C3H/He mice were sacrificed 7, 40, or 75 days after transplantation of BALB/c heart. They received 100 μg of either YN1/1.7 or KBA alone, or 50 μg each of both daily starting at the day of transplantation and lasting until the sixth day. Fresh spleen cells from the recipients were washed three times after lysis of red blood cells by 175 mM ammonium chloride. A standard 4-hour cytotoxicity assay was done with P815 cells labeled with ^{51}chromium as target cells (4 × 10^4 per well). Results are expressed as percent lysis. Data are indicated as means of triplicated samples. The experiment was repeated twice, and the results were consistent.

Treatment	Cardiac transplant	Days after surgery	Effector/target ratio	
			5	20
None	+	7	6.3	20.8
YN1/1.7	+	7	5.2	15.4
KBA	+	7	0.1	6.3
YN1/1.7 + KBA	+	7	1.2	6.7
YN1/1.7 + KBA	+	40	2.5	8.3
YN1/1.7 + KBA	+	75*	1.8	3.6
None	–		2.3	6.5

*The recipient mouse was transplanted with donor-syngeneic and third-party skin 8 days before the cytotoxic assay.

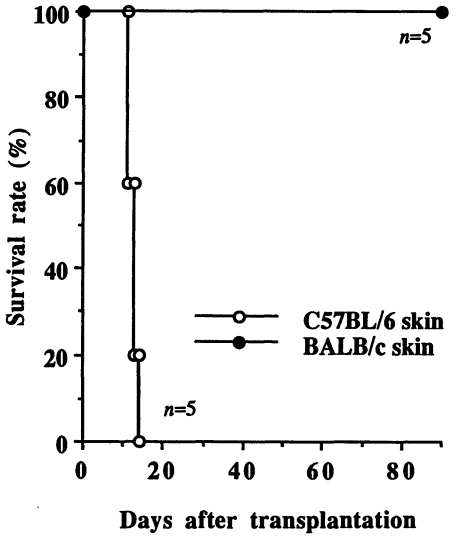

Fig. 2. Survival of skin grafts in C3H/He mice that accepted BALB/c mice heart for more than 65 days after a 6-day course of anti–ICAM-1/anti–LFA-1 treatment. Each mouse was transplanted with BALB/c and C57BL/6 full thickness body skin simultaneously. Third-party skin was rejected within 14 days, whereas cardiac donor-syngeneic skin was accepted indefinitely in all mice tested.

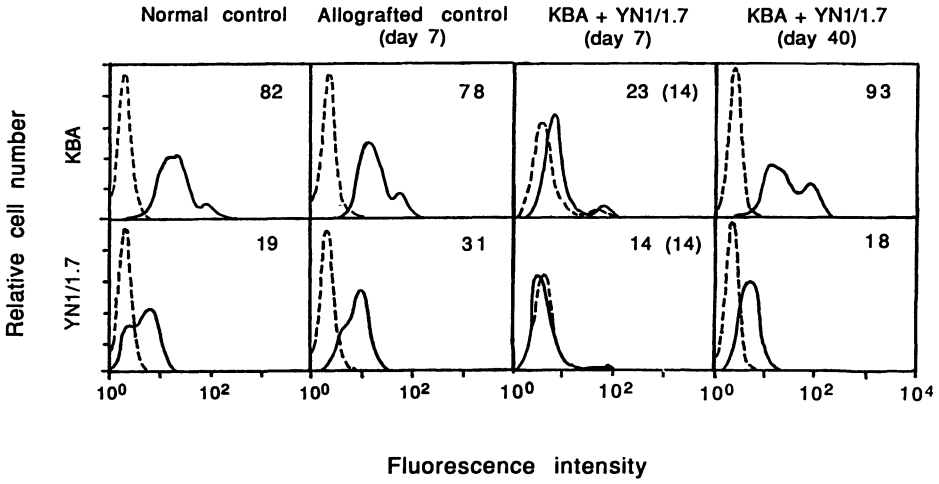

Fig. 3. FACS analysis of LFA-1 and ICAM-1 expression on splenocytes from allografted mice. Indirect immunofluorescent staining was performed with either KBA (anti-CD11a) or YN1/1.7 (anti–ICAM-1) as a primary antibody. Background staining with fluorescein isothiocyanate (FITC)–conjugated goat antibodies to rat Ig alone (dotted lines) was <1% unless otherwise noted in the parentheses. Profiles of stained cells are shown by solid lines, and percentages of positively stained cells are expressed by numbers. Mice were treated daily with the indicated MAbs for the first 6 days after transplantation except for the normal and allografted controls. Background staining in mice treated with nonspecific rat IgG in the same protocol was less than 1% at day 7, suggesting that the high background observed on day 7 in mice treated with MAbs was a result of the persistence of the administered MAbs binding to their antigens on the splenocytes.

spleen, and the Thy1.2$^+$, CD4$^+$, and CD8$^+$ subpopulations of splenocytes were not reduced at day 7 by the MAb treatment. However, flow cytometry (indirect immunofluorescence) analysis of LFA-1 and ICAM-1 expression on splenocytes of allografted mice indicated that the MAb treatment led to significant reduction of LFA-1– and ICAM-1–positive cells at day 7 after transplantation (Fig. 3). This down-regulation of the antigens may account for the inability to detect alloreactive CTL activity at this time (Table 2) and could be responsible for the induction of tolerance against alloantigens. However, the expression of LFA-1 and ICAM-1 was normal 40 and 75 days after transplantation, although alloreactive CTL activity was still not detected. Thus, the unresponsiveness is probably maintained by some mechanism other than down-modulation of LFA-1 and ICAM-1 molecules on allo responding cells.

The combination of both anti–ICAM-1 and antibody to CD11a (anti-CD11a) MAbs was required to induce tolerance. Each MAb used in this study alone can inhibit in vitro cell-mediated cytotoxicity (*10, 13*). However, our in vivo results showed that each MAb has only a modest effect on the prolongation of graft survival. Persistent acceptance of the grafts was achieved only by simultaneous administration of these two MAbs. Although further investigations are needed, the redundancy of adhesion pairs may partly account for this synergism. LFA-1 has at least three ligands, ICAM-1, ICAM-2 (*18*), and a third, unknown ligand (*19*). In addition, ICAM-1 has other counter-receptors, Mac-1 (*20*) and CD43 (*21*). Mac-1 and LFA-1 bind to discrete domains on ICAM-1 (*20*). However, the involvement of these adhesion molecules in allograft rejection and tolerance induction remains to be determined. Whatever the mechanism, the ICAM-1 and LFA-1 interaction is important in the pathogenesis of allograft rejection. This mode of immunosuppression could perhaps be applied to individuals undergoing organ transplantation.

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Isolation of Two Genes That Encode Subunits of the Yeast Transcription Factor IIA

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The yeast transcription factor IIA (TFIIA), a component of the basal transcription machinery of RNA polymerase II and implicated in vitro in regulation of basal transcription, is composed of two subunits of 32 and 13.5 kilodaltons. The genes that encode these subunits, termed *TOA1* and *TOA2*, respectively, were cloned. Neither gene shares obvious sequence similarity with the other or with any other previously identified genes. The recombinant factor bound to a TATA binding protein–DNA complex and complemented yeast and mammalian in vitro transcription systems depleted of TFIIA. Both the *TOA1* and *TOA2* genes are essential for growth of yeast.

IN ADDITION TO RNA POLYMERASE II (RNAPII), initiation of mammalian mRNA synthesis requires at least eight general transcription initiation factors, termed TFIIA, B, D, E, F, G, H, and J. These general factors are required for transcription from a core promoter containing a TATA element and a transcription initiation site (*1, 2*). The mechanism by which the general factor TFIIA acts is not well understood. Although some fractionated transcription systems lack a TFIIA requirement (*3*), the most highly purified human systems have a strong TFIIA dependence (*2, 4*). In a fractionated yeast transcription system, yeast TFIIA (yTFIIA) is required for high levels of transcription promoted by an acidic transcriptional activator (*5*). Several results suggest that TFIIA may perform an important regulatory function by blocking the action of inhibitors of transcription (*2, 4, 6*). While not itself a DNA binding protein, TFIIA tightly binds to a TATA binding protein (TBP)–DNA complex (*5, 7, 8*). Below, we refer to the TATA binding protein as TBP and refer to TFIID as the multisubunit transcription factor that contains TBP (*9*).

The reported subunit composition of TFIIA from different sources is variable (*2, 10*). Purified yTFIIA consists of two polypeptides of 32 and 13.5 kD (*5*), which we term TOA1 and TOA2, respectively. Together, these two polypeptides can bind to a TBP-DNA complex and restore transcription to both yeast and mammalian in vitro transcription systems depleted of TFIIA. Neither subunit alone shows any activity in transcription or TBP-DNA binding (*5*). To better understand the function of TFIIA, we have cloned the genes encoding both subunits of the yeast factor.

We purified TFIIA from yeast using a TBP-DNA affinity column (*5*), and sequenced tryptic peptides generated from both the TOA1 and TOA2 subunits (*11*) (Fig. 1). The peptide sequences were used to design degenerate polymerase chain reaction (PCR) primers for amplification of small segments of each gene (*12*). These PCR products were then used as probes in screens of yeast genomic libraries to isolate the entire coding sequences for *TOA1* and *TOA2* (*13*). *TOA1* consisted of an open

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