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THE COMPLEMENT DEPENDENT CYTOTOXICITY (CDC) IMMUNE EFFECTOR MECHANISM CONTRIBUTES TO ANTI-CD154 INDUCED IMMUNOSUPPRESSION

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Abstract

Background—In many situations, anti-CD154 (CD40 ligand) monoclonal antibody (mAb) treatment is very potent in producing allograft tolerance. In accordance to our previously reported results, combined donor specific transfusion (DST)[3] plus anti-CD154 mAb (MR1) treatment enables the permanent engraftment of DBA/2 (H-2^{d}) islets into B6AF1 (H-$2^{b/kd}$) recipients in all cases. It has been widely assumed that the MR1 anti-154 is a noncytolytic neutralizing mAb, and it exerts immune suppressive effects by blockade of CD40/ CD154 signal pathway. In this study, we sought to test the role of complement dependent cytotoxicity (CDC) immune effector mechanism in MR1 anti-CD154 induced immunosuppression.

Methods—We have evaluated the contributions of CDC in the context of the potent tolerizing effects of DST plus anti-CD154 mAb treatment regiment in recipients of islet allografts. We have used CD40 knockout (KO) mice and complement C5 deficient mice DBA/2 as islet allograft recipients as well as cobra venom factor (CVF), a complement blocker, treatment.

Results—The absence of direct and indirect CD40/CD154 pathway signals does not prevent islet allograft acute rejection. Interestingly, MR1 anti-CD154 induces islet allograft tolerance in the absence of CD40/ CD154 pathway. In a wild-type major histocompatibility complex (MHC) mismatched strain combination, DST results in accelerated islet allograft rejection. Combination of DST and MR1 anti-CD154 treatment prevents presensitization and permits permanent engraftment. However, administration of CVF abolishes the tolerance induction. Moreover, DST plus MR1 anti-CD154 regiment, a potent tolerizing therapy, does not prevent acute islet allograft rejection when complement C5 deficient DBA/2 mice are used as recipients. Thus, the mechanisms of the tolerizing effects by MR1 anti-CD154 are not limited to blockade of CD40/ CD154 signals. The CDC immune effector mechanism contributes to MR1 anti-CD154 induced immunosuppression.

Robust activation of naive T cells to alloantigen requires two signals. The first signal is delivered when the T-cell receptor (TCR) engages the major histocompatibility–antigen complex (MHC-Ag), and the second costimulatory signal is delivered when the Ag-stimulated T cells interact with co-stimulatory molecules on antigen presenting cells (APC) (1). On the other hand, failure to deliver a costimulatory “second” signal with Ag presentation induces a state of T-ell anergy (2).

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There are at least two potent costimulatory signaling pathways that are essential for normal development and maintenance of immunity (3, 4). In one system, APC cell surface molecules CD40 interact with CD40 ligand (CD154) proteins expressed on activated T cells. In the B7/CD28 pathway, APC cell surface B7.1 (CD80) and B7.2 (CD86) molecules deliver a costimulatory signal by interacting with the CD28 cell surface proteins expressed on resting T cells (1, 5). Although the CD40/CD154 and the B7/CD28 pathways are interrelated, each of these two pathways serves as a critical, independent regulator of T-cell dependent immune responses (6).

The CD40 pathway plays a critical role in allograft rejection, as inhibition of this pathway with an anti-CD154 monoclonal antibody (mAb) dramatically prolongs murine islet and cardiac allograft (7, 8) as well as primate monkey allograft survival (9). In accordance with our previously reported results, combined donor specific transfusion (DST) plus anti-CD154 mAb (MR1) treatment enables the permanent engraftment of DBA/2 (H-2^{d}) islets into B6AF1 (H-$2^{b/kd}$) recipients in all cases (10).

Although it has been widely assumed that the MR1 anti-CD154 is a noncytolytic neutralizing mAb and that it exerts immune suppressive effects by blockade of CD40/CD154 signal pathway, recent studies suggested that the potent immunosuppressive effects of MR1 anti-CD154 are not limited to the blockade of CD40/CD154 signal pathway (11). Because our recent studies indicate the importance of trimming alloreactive T-cell clone size in the induction of allograft tolerance (12, 13), in this study, we sought to test the role of complement dependent cytotoxicity (CDC) immune effector mechanism in MR1 anti-CD154 induced immunosuppression.

As was consistent with our previous report, the combination of DST and MR1 anti-CD154 treatment induced DBA/2 (H-2^{d}) islet allograft tolerance in all B6AF1 (H-$2^{b/kd}$) recipients. The benefit of this treatment, however, is almost totally negated by concomitant administration of cobra venom factor (CVF), a reagent that depletes complement (Table 1). To further pursue the possibility that a complement dependent action of MR1 may be required for optimal therapeutic effects, we have compared the effects of DST plus MR1 in experiments that use B6AF1 donors and either Balb/C (H-2^{d}) or C5 complement deficient DBA/2 recipients (14). In both models, a semi-allogeneic barrier to engraftment is present. In BALB/c recipients, treatment with DST plus anti-CD154 mAb universally produces permanent engraftment (Table 1). In complement C5 deficient DBA/2 recipients, only one of four recipients were permanently engrafted (Table 1). To confirm that the optimal benefit conferred by MR1 therapy involves additional mechanisms other than costimulation blockade, we evaluated the impact of the total elimination of CD40 triggered CD154 signals by using CD40 deficient (knockout [KO]) 129 (H-2b) and Balb/C (H-2d) as donors and recipients, respectively (Table 1). In this system, CD40 triggered costimulation cannot take place through either the direct or indirect pathways of alloantigen presentation. As expected, DST plus MR1 ensures permanent engraftment of wild-type 129 islets into wild-type Balb/C recipients (Table 1). In stark contrast, DST treatment does not prevent acute rejection when both CD40KO donors and recipients are employed (Table 1). Interestingly, MR1 plus DST results in the permanent engraftment of CD40KO islet allografts in three out of six CD40KO recipients (Table 1). Hence, the full tolerizing effects of MR1 anti-CD154 mAb require the involvement of supplementary mechanisms other than costimulation blockade. In addition to the qualitative changes on T-cell activation (immune deviation) imposed by anti-CD154 treatment, an antibody and complement mediated reduction in the mass of alloreactive T cells may also be required to achieve permanent engraftment/tolerance.

In collaboration with Larry Turka's lab, we reported the importance of preserving apoptosis of activated T cells for establishing allograft tolerance in MHC mismatched models (12, 13).

Our previous work indicated that to establish transplantation tolerance with treatments based on Th1/Th2 immune deviation and anergy, the vast pool of T cells responding to allogeneic MHC molecules had to be trimmed. Although this work has often been interpreted as showing a need for apoptosis in tolerance induction, in our opinion, this interpretation may be too narrow because apoptosis is only one of several means available to kill T cells. Our present report fully supports and adds breadth to this notion that deletional as well as anergy and immune deviation mechanisms are absolutely crucial to the induction of peripheral allograft tolerance. We believe the current work has significant practical as well as theoretical significance.

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Table 1

The contributions of CDC in the context of the potent tolerizing effects of DST plus anti-CD154 mAb treatment regimen in recipients of islet allografts

Donor	Recipients	Treatment	Graft survival
DBA/2	B6AF1	untreated	9, 11, 13, 15, 17
DBA/2	B6AF1	DST[a] + MR1[b]	>150 n=10
DBA/2	B6AF1	DST[a] + MR1[b] + CVF[c]	15, 15, 19, 29, 32
B6AF1	DBA/2	untreated	13, 14, 15, 20
B6AF1	DBA/2	DST[a]	13, 13, 14, 15, 15
B6AF1	DBA/2	DST[a] + MR1[b]	20, 24, 24, 40, >120
B6AF1	Balb/c	untreated	9, 11, 13, 15, 17
B6AF1	Balb/c	DST[a]	7, 7, 8, 9, 10, 12
B6AF1	Balb/c	DST[a] + MR1[b]	>60, >60, >60, >60
129 CD40KO	Balb/c CD40KO	untreated	9, 12, 13, 17
129 CD40KO	Balb/c CD40KO	DST[a]	6, 6, 7, 9, 10
129 CD40KO	Balb/c CD40KO	DST[a] + MR1[b]	8, 19, 22, >120, >120, >120

[a]DST consisted of 0.25 mL fresh donor whole blood administered 28 days before transplantation.

[b]MR1 anti-CD154 mAb was given at a single dose of 0.5 mg/mouse intravenously 28 days before transplantation.

[c]CVF was given at a dose of 60 units/kg intraperitoneally 28 days before transplantation followed by 20 units/kg per day for 14 consecutive days.

DST, donor specific transfusion; CVF, cobra venom factor.