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Tolerogenic Dendritic Cells and Myeloid-Derived Suppressor Cells: Potential for Regulation and Therapy of Liver Auto- and Alloimmunity

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Abstract

Organ transplantation is now established as an accepted treatment for end-stage liver disease, acute fulminant hepatic liver failure and hepatocellular carcinoma. While early graft acceptance rates have increased markedly due to improved immunosuppressive drug regimens, rates of late graft failure remain largely unchanged. Recent findings suggest that, in addition to alloimmunity, chronic rejection of liver allografts may also reflect de novo autoimmune hepatitis or recurrence of pre-existing hepatic autoimmune disease. Dendritic cell (DC)-based therapy is a promising experimental approach to promotion of transplant tolerance and the treatment of autoimmune diseases. Newly-emerging evidence also demonstrates the potential efficacy of myeloid-derived suppressor cells (MDSC) in the antigen (Ag)-specific regulation of T cell responses. Herein, we discuss current understanding of liver autoimmunity post transplantation, along with current approaches for the development of tolerogenic DC, and the potential use of MDSC for the development of stable, Ag-specific tolerance.

Keywords

Autoimmunity; dendritic cells; liver; myeloid-derived suppressor cells; transplantation

Introduction

The liver is a unique site for immune regulation, striking a delicate balance between tolerance and immunity (Crispe, 2009; Carambia and Herkel, 2010). It is a prime candidate for persistent inflammation given that it resides downstream of the gut and is continually exposed to endotoxin and other microbial degradation products that reach the liver via portal blood. Further, the liver is responsible for drug metabolism and detoxification and is susceptible to cellular destruction by alcohol metabolism. These processes may cause exposure to neo-antigens (Ags) and the potential onset of autoimmune disease. Paradoxically, the liver is also regarded as a site of unique immune privilege and immune

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tolerance, particularly in the context of transplantation. In humans, clinical operational tolerance has been described most frequently following liver transplantation (Castellaneta et al., 2010) and liver transplants can protect other organ grafts from the same donor from rejection (Rasmussen et al., 1995). Spontaneous acceptance of liver allografts between complete major histocompatibility complex (MHC)-mismatched donors and recipients has been described in both mouse and rat models (Farges et al., 1994; Qian et al., 1994). Despite the inherent tolerogenicity of the liver, viral infections, such as hepatitis C, as well as autoimmune disorders, that include autoimmune hepatitis (AIH), primary biliary cirrhosis, primary sclerosing cholangitis and halothane hepatitis do occur. While the incidence of hepatic autoimmunity is less prevalent than extrahepatic autoimmune disorders, such as type-I diabetes, rheumatoid arthritis, and multiple sclerosis, the consequences of liver autoimmunity are severe and may necessitate transplantation.

In an effort to move away from global immune suppression, that can lead to adverse side effects and susceptibility to infection and malignancy, cellular therapies are increasingly the subject of investigation to achieve Ag-specific immune regulation. Dendritic cells (DC) are bone marrow (BM)-derived professional Ag-presenting cells (APC), with inherent tolerogenic ability. Pharmacological modulation of DC with anti-inflammatory cytokines and agents such as rapamycin, dexamethasone and Vitamin D has been used in animal models to induce “semi-mature,” “alternatively-activated,” or maturation-resistant DC, able to suppress T cell function in transplantation and autoimmune disease (Morelli and Thomson, 2007; Turnquist et al., 2007; Anderson et al., 2008; Hilkens et al., 2010). An emerging cell population of interest, myeloid-derived suppressor cells (MDSC) (Gabrilovich and Nagaraj, 2009), may also offer opportunities for the development of cellular therapy. MDSC are a heterogeneous population of immature myeloid cells, originally shown to accumulate at the site of tumors. These cells can potently suppress T cell responses by Ag-specific mechanisms (Nagaraj et al., 2007). Cell-based therapies using stably-immature DC or MDSC represent promising avenues for the development of Ag-specific tolerogenic strategies targeted at prevention of allograft rejection and inhibition/reversal of autoimmune disease.

Autoimmune disease following liver transplantation

Liver transplantation is an accepted treatment for end-stage liver disease, hepatocellular carcinoma, and fulminant hepatic liver failure. In the early years of human liver transplantation, acute rejection was frequent, occurring in up to 75% of patients (Ormonde et al., 1999). However, with the advent of safer and more effective immunosuppressive agents, the rate has fallen to roughly 30% of allograft recipients and graft survival in adults is now >82% and 68% at 1 and 5 years, respectively (Ormonde et al., 1999; Waki, 2006). However, successful prevention of acute rejection has greatly increased the number of patients experiencing long-term complications, including chronic rejection, recurrence of disease, de novo AIH and cancer (Vergani and Mieli-Vergani, 2002). Recent evidence suggests that the de novo occurrence of autoimmune disease post-transplant may be a mechanism underlying chronic rejection. It was reported initially in pediatric transplant recipients, who developed graft dysfunction at a median of two years post-transplant, along with anti-nuclear, anti-smooth muscle and anti-liver-kidney microsome autoantibodies (Kammer et al.) (Kerkar et al., 1998). These patients also had high concentrations of serum IgG and histological features of AIH, such as periportal infiltration of plasma cells and lymphocytes. Interestingly, the majority of these patients responded to prednisolone and azathioprine, as prescribed for AIH, with a decrease in cyclosporine A or tacrolimus that are standard anti-rejection therapies. De novo AIH has been further reported in pediatric patients, as well as in adult liver transplant recipients (Gupta et al., 2001; Salcedo et al., 2002), with the presence

of autoAbs correlating negatively with graft function, and graft and patient survival (Venick et al., 2007).

Mechanisms of de novo AIH onset

While de novo AIH is a well-recognized clinical problem, its etiology, like that of most autoimmune liver diseases, is largely unknown. This is due mainly to the inherent tolerogenicity of the liver, which makes it difficult to establish reliable small animal models of AIH. Recently however, new models have been developed that are shedding light on the possible causes of AIH (Christen et al., 2009). It has been speculated that high dose immune suppression used to combat acute rejection can promote the development of chronic allograft injury through the development of autoimmunity. Moreover, studies in mouse models have revealed that immunosuppressive treatment with cyclosporine A or rapamycin inhibits the deletion of self-reactive T cell receptor (TCR) $V\beta^+$ T cells, which are then poised to induce immune responses to self Ags (Gao et al., 1988; Bucy et al., 1993).

Molecular mimicry, the structural similarity between viral and host proteins (Rose and Mackay, 2000), has been implicated as another possible etiology of AIH. The theory of molecular mimicry has particular relevance, because autoAbs to cytochrome P450 epitopes are found in patients who develop de novo AIH post transplant (Lohse et al., 1999). These epitopes are structurally similar to hepatitis C viral epitopes (Kammer et al., 1999). It was shown recently that infection of mice with liver-tropic adenovirus expressing the human autoAg cytochrome P450 2D6 (CYP2D6) could break self tolerance and induce AIH characterized by anti-CYP2D6 Abs, inflammatory cell infiltration and hepatic fibrosis (Holdener et al., 2008). In this model, viral infection was essential for the development of AIH, as injection of recombinant CYP2D6 with a number of different adjuvants failed to induce disease. Of note is that injection of CYP2D6 together with complete Freund's adjuvant (CFA) induced autoAb production, but was not sufficient to induce liver damage or elevated alanine aminotransferase, or aspartate aminotransferase indicating that autoAb alone is not sufficient to induce liver damage. Notably, the role of autoAbs in the clinical manifestation of AIH post liver transplant has not been firmly established, and it is likely that cell-mediated immune responses play a major role (Nakano et al., 2008).

The onset of hepatic autoimmunity has also been linked to the metabolism of halogenated anesthetics. Drug-induced hepatitis accounts for nearly 13% of acute liver failure in the United States and is the third most common indication for liver transplantation (Masubuchi et al., 2003). A mouse model of AIH has recently been developed, based on the hypothesis that drug-induced hepatitis is caused by covalent modification of self proteins, specifically the endoplasmic reticulum protein and CYP2E1, by drug-haptens. The Ag used in this model is trifluoroacetyl chloride (TFA)-modified S100 protein, which itself has been shown to induce AIH (Lohse et al., 1990). Immunization with TFA-S100 in CFA results in production of autoAbs against the TFA hapten, the S100 protein, and the self protein CYP2E1 (Njoku et al., 2005). This model exhibits clinical features of drug-induced hepatitis, such as mast cell and eosinophilic infiltration, decreased numbers of Kupffer cells, and CYP2E1-specific IgG4 autoAbs (Njoku et al., 2005; Njoku et al., 2006). Autoreactive T cells specific for the TFA hapten and CYP2E1 have been demonstrated, along with adoptive transfer of disease by $CD4^+$ T cells (Njoku et al., 2009). As this model is relatively new, various components of the immune response, such as the role of hepatic APC, remain to be elucidated.

There is significant heterogeneity in the clinical features of AIH, suggesting that the underlying etiologies among patients are also diverse and heterogeneous. While the specific etiology of clinical de novo AIH is largely unknown, understanding the mechanisms

underlying AIH in animal models is of critical importance to establishing treatment modalities for patients experiencing chronic rejection post-liver transplantation.

Use/Targeting of DC for Ag-specific therapy for allograft rejection and autoimmune disease

DC are a rare, heterogeneous population of professional BM-derived APC, distributed in virtually all organs and in the circulation. These cells take up and process Ag for presentation to naïve or memory T cells, initiating DC-T cell crosstalk required for the activation and expansion of Ag-specific effector T cells. Although mature DC function as potent initiators of adaptive immunity, there is also evidence that DC play a powerful role in the maintenance of peripheral tolerance. This regulatory function has been shown to be dependent on the maturation status and subtype of the DC. For example, plasmacytoid DC (pDC) were shown recently to mediate oral tolerance, by suppressing Ag-specific CD8⁺ T cell function in the liver (Goubier et al., 2008). Further, Ab-mediated depletion of pDC abrogated the induction of oral tolerance. Currently, the therapeutic potential of pDC remains difficult to harness due to the recent identification of new subsets and differentiation states of pDC, which makes defining a discrete population of regulatory pDC difficult (Hadeiba et al., 2008; Segura et al., 2009).

On the other hand, conventional myeloid DC represent a promising population for cellular therapy, due to the development of culture methods for generating large numbers of these cells (Caux et al., 1997) that can be pharmacologically manipulated *in vitro*. Myeloid DC have already been used for development of Ag-specific T cells for clinical anti-cancer therapy (Palucka et al., 2009; Palucka et al., 2010). They are now being explored for Ag-specific immune regulation in transplantation and autoimmunity (Figdor et al., 2004; Bruder et al., 2005; Morelli et al., 2007; Marin-Gallen et al., 2009; Hilkens et al., 2010). It was believed initially that the key to developing tolerogenic DC would be the induction of stably immature cells that express low levels of costimulatory molecules (CD80 and CD86) and produce low levels of IL-12 and high levels of IL-10. Recently however, evidence has emerged that DC maturation status alone does not define tolerogenic or immune-activating DC. For instance, in a murine model of type-I diabetes, CD86^{hi} BM-derived DC loaded with a pancreatic islet beta cell-specific peptide expanded Ag-specific CD4⁺CD25⁺ regulatory T cells (Treg) (Tarbell et al., 2004). Further, costimulation is required for Forkhead box protein-3⁺ (Foxp3⁺) Treg expansion in the thymus (Tai et al., 2005). In addition, because immature DC have weak migratory properties, induction of the expression of chemokine receptors, such as CCR7 (the receptor for CCL19 and CCL21) is required to ensure that these DC home to lymphoid tissue in order to affect T cell activation/expansion (Anderson et al., 2009). Therefore, functional, as well as phenotypic assessment of DC is essential in determining their therapeutic potential.

Several pharmacological agents have been used for DC modification with promising results in animal models of allograft rejection and autoimmune disease. Thus, for example, maturation-resistant BM-derived DC have recently been generated by *in vitro* conditioning with rapamycin (RAPA-DC) (Turnquist et al., 2007). These cells specifically suppress the development of CD4⁺ effector T cells, while promoting Ag-specific development of CD4⁺ Foxp3⁺ Treg. Further, when recipient-derived, donor allo-Ag-pulsed RAPA-DC are infused into heart allograft recipients given a short post-transplant course of rapamycin, they induce indefinite graft survival (Turnquist et al., 2007).

Generation of DC from human peripheral blood mononuclear cells in the presence of dexamethasone, active VitaminD3 (1 α , 25-dihydroxy vitamin D3) and lipopolysaccharide (LPS), (“alternatively-activated”) DC are able to skew T cells toward a more regulatory,

IL-10-producing phenotype, along with a lack of IL-12p70 production (Anderson et al., 2008). Of further interest is that in the presence of LPS, these cells develop a 'semi-mature' phenotype and present Ag efficiently, with upregulated surface CD80 and CD86 (Anderson et al., 2009). More importantly, LPS causes upregulation of CCR7 and these DC can migrate more efficiently toward CCL19 in a transwell system. Tolerogenic DC developed in the presence of rapamycin also retain surface CCR5 and CCR7 expression, and home efficiently to secondary lymphoid organs *in vivo* as demonstrated in allogeneic BM cell transplantation (Reichardt et al., 2008). This migratory capacity is essential for the efficacy of tolerogenic DC therapy, as once these cells are infused, they must be able to home to T cell areas of secondary lymphoid tissue in order to exert their tolerogenic functions, as in organ transplantation (Garrod et al., 2006).

DC-based therapies have also shown promise for the treatment of experimental autoimmune disease (Thomson and Robbins, 2008; Hilkens et al., 2010). For example, DC generated in the presence of the drug BAY 11-7082, which blocks nuclear factor (NF)- κ B signaling through inhibition of I κ B phosphorylation, inhibit established arthritis in a mouse model, in an Ag-specific manner (Martin et al., 2007). This strategy suppressed clinical and histological disease severity after full disease onset. Since BAY 11-7082 is an irreversible NF- κ B inhibitor, these conditioned DC are unlikely to display a strong response to danger signals, such as LPS, or CpG, and therefore carry less danger of becoming fully mature and able to induce inflammation once administered *in vivo*.

Further approaches include the use of liposomes for delivery of Ag to DC *in situ*, combined with immunosuppressive agents *in vivo*, to obviate the need for *ex vivo* generation of tolerogenic DC. One of the early demonstrations of the efficacy of liposome-mediated delivery of therapeutic agents was in the context of experimental autoimmune encephalomyelitis (EAE). In this model, liposome-encapsulated autoAg (myelin basic protein) delivered to rats by intracardiac injection reduced the clinical disease score and myelin basic protein-specific T cell proliferation (Stein et al., 1993). In keyhole limpet hemocyanin (KLH)-immunized mice, small interfering (si)RNA to CD40 encapsulated into liposomes could be directed to DC *in vivo* using CD205 (DC surface marker)-specific Abs. In this way, the authors were able to specifically target the siRNA to DC and to silence CD40 expression in the liver, spleen and kidney, and also to suppress KLH-specific T cell proliferation (Zheng et al., 2009). Mechanisms of T cell suppression by pharmacologically-modified DC are summarized in Figure 1A.

A similar approach based on delivery of antisense oligonucleotides for the downregulation of CD40 has been shown to attenuate arthritis in a mouse model. Thus, liposome-mediated delivery of both ovalbumin (OVA) and NF κ B inhibitors to DC resulted in expansion of OVA-specific Treg and suppressed OVA-induced arthritis (Capini et al., 2009). This approach is particularly applicable to autoimmune diseases caused by known Ags, such as rheumatoid arthritis, and type-I diabetes. It may also have implications for therapy of transplant rejection by loading donor-derived allo-Ag into liposomes for expansion of donor-specific T reg. As our understanding of the Ags responsible for AIH increases, similar treatment strategies using liposomes loaded with auto-, as well as allo-Ag may provide a means by which to subvert chronic liver allograft rejection.

Use/Targeting of MDSC for Ag-specific prevention of allograft rejection and autoimmune disease

MDSC are a heterogeneous population of cells composed of macrophage, granulocyte and DC precursors. These regulatory myeloid cells accumulate at tumor sites and have been shown to limit the efficacy of cancer therapy (Gabrilovich, 2004). Specifically, in

hepatocellular carcinoma patients, as well as in mouse tumor models, MDSC have been shown to accumulate at the tumor site, where they inhibit both natural killer cell and effector T cell activity (Hoechst et al., 2009; Ilkovitch and Lopez, 2009). In humans, MDSC are characterized as CD33⁺CD11b⁺CD14⁻ cells, that do not express markers such as MHC class II that are expressed on mature cells of myeloid origin (Ochoa et al., 2007). In mice, they express the integrin CD11b and the myeloid differentiation Ag Gr1 (Nagaraj and Gabrilovich, 2008). These cells comprise <5% of total cells in the spleen and liver, and roughly 20% of BM cells in healthy mice (Ilkovitch et al., 2009). However, in various disease settings, such as cancer, sepsis, autoimmunity and transplantation, these cells accumulate in high numbers, and are capable of suppressing T cell-mediated responses (Delano et al., 2007; Zhu et al., 2007; Dugast et al., 2008; Ostrand-Rosenberg and Sinha, 2009).

Based on surface marker expression, mouse MDSC can be further divided into a CD11b⁺Ly6C⁺ monocytic subset and a CD11b⁺Ly6G⁺ granulocytic subset. It is important to consider that these different subsets have been shown to have differential functions in various disease settings. For example, CD11b⁺Ly6C⁺ MDSC were shown to inhibit OVA-specific T cell proliferation by a nitric oxide (NO)-specific mechanism, while the CD11b⁺Ly6G⁺ population had no suppressive capacity (Dietlin et al., 2007). By contrast, in a mouse tumor model, both the granulocytic and monocytic subtypes were able to suppress OVA-specific T cell responses, but did so via distinct mechanisms. The monocytic (Ly6C^{hi}) population mediated suppression by a NO-dependent mechanism, while suppression by the granulocytic (Ly6G^{hi}) subpopulation required IFN γ (Movahedi et al., 2008). Therefore, in order for these cells to be viable as cellular therapies, the specific subtype with suppressive function must be identified for each pathological condition.

Relatively little is known regarding the function of these cells in autoimmune diseases, however their numbers have been shown to increase dramatically in several models of autoimmunity. In a mouse model of autoimmune uveoretinitis, a population of CD11c⁺Ly6G⁻ cells with the capacity to suppress CD4⁺ T cell proliferation has been described (Kerr et al., 2008). Further, in a mouse model of chronic contact eczema, induced for the treatment of autoimmune alopecia areata, CD11b⁺Gr1⁺ cells infiltrate the skin, lymph node and spleen (Marhaba et al., 2007). In this model, CD11b⁺Gr1⁺ cells inhibit CD4⁺ and CD8⁺ T cell proliferation by downregulating TCR ζ -chain. MDSC have also been shown to accumulate in a mouse model of inflammatory bowel disease (IBD), and can prevent the onset of disease when co-transferred with peptide-specific CD8⁺ T cells (Haile et al., 2008). The authors also report that arginase-expressing MDSC with suppressive function accumulate in the peripheral circulation of patients with IBD.

In EAE, CD11b⁺Gr1⁺ MDSC have been shown to accumulate in the spleen (Zhu et al., 2007). These MDSC could be further divided phenotypically based on the degree of Gr1 expressed, further highlighting the heterogeneous nature of this population. Importantly, the CD11b⁺Ly6C^{hi} subset suppressed CD4⁺ and CD8⁺ T cells, as well as polyclonally activated CD4⁺ T cells by inducing apoptosis. This monocytic subset has also been shown to suppress T cell activation through production of arginase-1 and NO synthase-2 (Kusmartsev et al., 2005; Zhu et al., 2007). It is important to note that this subset of cells makes up only ~7% of CD11b⁺ cells in the spleen of EAE mice, whereas the Ly6C^{int}Ly6G⁺ population comprises almost 75% of CD11b⁺ cells, but did not exert any suppressive function (Zhu et al., 2007). Therefore, the limited numbers of these cells may not have been sufficient to suppress disease. Nevertheless, they constitute new candidates for the development as cellular therapeutic agents.

Evidence from transplant models also demonstrates that MDSC can suppress effector T cells. In a rat kidney allograft model, the number of MDSC that accumulated in the graft and in the circulation correlated with graft survival (Dugast et al., 2008). These cells expressed CD80 and CD86 and could inhibit bulk T cells in MLR through inhibition of IL-2 uptake. This suppression required production of inducible (i)NO synthase by MDSC and MDSC-T cell contact, but was not Ag-specific. Interestingly, MDSC also reduced proliferation of Foxp3⁺ Treg by 50%. However, Treg were not required for suppression of effector T cells, as inhibition of iNOS produced by MDSC was sufficient to restore T cell proliferation. A recent study also demonstrated that adoptive transfer of non-Ag-specific suppressive MDSC elicited upon induction of systemic endotoxin tolerance could prolong skin allograft survival in a heme-oxygenase-1 (HO-1) dependent manner (De Wilde et al., 2009). Mechanisms of T cell suppression by MDSC are presented in Figure 1B. Further, conditional deletion of Src homology 2-containing inositol-5'-phosphatase (SHIP) results in the expansion of MDSC, and protects from lethal graft-versus-host disease by deficient priming of allogeneic T cells (Paraíso et al., 2007). The known mechanisms of MDSC-mediated T cell suppression in models of autoimmunity and transplantation are summarized in Table 1.

MDSC thus represent a promising population in the development of tolerogenic cell therapies, given that (like tolerogenic DC) they can suppress T cell proliferation and function in an Ag-specific manner. Determination of factors that can induce proliferation of these cells is likely to prove important. However, in many instances, MDSC are not terminally differentiated, which leaves open the possibility that these cells can mature *in vivo* into immune-activating DC or macrophages. Studies in mouse models, as well as in cancer patients, have demonstrated that all-trans retinoic acid (ATRA) can induce the differentiation of MDSC into mature myeloid cells (Nefedova et al., 2007). Incubation of mouse tumor-derived MDSC or human peripheral blood MDSC with ATRA upregulates glutathione, which in turn, significantly upregulates surface CD11c, F4/80 and MHC class II. The production of reactive oxygen species (ROS) is an important mechanism by which MDSC suppress T cell responses in cancer. Inhibition of ROS by scavenging of H₂O₂ induces MDSC differentiation into macrophages (Kusmartsev and Gabrilovich, 2003). Therefore, as glutathione is a potent antioxidant, high levels can induce MDSC differentiation into mature inflammatory APC. As the mechanisms of differentiation of MDSC are further elucidated, approaches harnessing these cells to induce Ag-specific tolerance, can be devised for the treatment of autoimmune disease and transplant rejection.

Conclusion

There is evidence that both experimental autoimmune disease and allograft rejection can be controlled in an Ag-specific manner by *in situ* targeting of DC, or the adoptive transfer of tolerogenic DC. MDSC have also recently emerged as regulatory cells in certain autoimmune disease models and in transplantation. Cell-based therapies, based on DC or MDSC, may offer potential for the prevention or treatment of autoimmune liver disease, including its occurrence post-transplant in recipients of liver allografts.

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Abbreviations

Abs antibodies

Ag	antigen
AIH	autoimmune hepatitis
APC	antigen- presenting cell
BM	bone marrow
DC	dendritic cell
Foxp3	forkhead box protein-3
MDSC	myeloid-derived suppressor cell
MHC	major histocompatibility complex
NF	nuclear factor
NO	nitric oxide
OVA	ovalbumin
RAPA-DC	rapamycin-conditioned DC
siRNA	small interfering RNA
TCR	T cell receptor
TFA	trifluoroacetyl chloride
Treg	regulatory T cell

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Fig. 1.

A) Strategies for generation of tolerogenic DC through treatment with 1) liposomes containing siRNA and/or antigen, 2) Dexamethasone (Dex), VitD3, and LPS, and 3) rapamycin. Liposomes containing siRNA to co-stimulatory molecules, or cytokine mRNA can inhibit T cell responses through the restriction of activation signal 2 (costimulatory molecules- CD80/86) and 3 (pro-inflammatory cytokine production). Pharmacological modification with Dex, VitD3 and LPS induces upregulation of CCR7, required for migration to draining lymph nodes as well as CD80 and CD86, but also induces production of regulatory IL-10. Rapamycin-conditioned DC (RAPA-DC) express surface CCR5 and CCR7. RAPA-DC can promote tolerance through inducing the expansion of antigen-specific regulatory T cells. **B)** Myeloid-derived suppressor cells (MDSC) produce various mediators, such as arginase-1, nitric oxide and nitric oxide synthase, which inhibit T cell responses through modification of the ζ -chain TCR, inhibition of IL-2 uptake by T cells and induction of T cell apoptosis.

Table I

Mechanisms of T cell suppression by MDSC in autoimmune disease and transplantation

Model	MDSC phenotype	Mechanism of T cell suppression	Reference(s)
Experimental autoimmune uveoretinitis	CD11b ⁺ Gr1 ^{int}	NO production, Foxp3 ⁺ T cell expansion	(Liversidge et al., 2002; Kerr et al., 2008)
Alopecia areata	CD11b ⁺ Gr1 ^{int/hi}	Downregulation of T cell ζ-chain	(Marhaba et al., 2007)
Inflammatory bowel disease	CD11b ⁺ Gr1 ⁺	nitric oxide synthase 2, arginase production	(Haile et al., 2008)
Experimental autoimmune encephalomyelitis	CD11b ⁺ Ly-6C ^{hi}	NO production, T cell apoptosis	(Zhu et al., 2007)
Rat kidney allograft	CD11b ⁺ CD80/86 ⁺ NKRP-1 ⁺ ^I	Inhibition of IL-2 consumption, nitric oxide synthase production	(Dugast et al., 2008)
Mouse skin allograft	CD11b ⁺ Gr1 ⁺	Heme oxygenase-1 production	(Zhang et al., 2008; De Wilde et al., 2009)

^I Natural Killer Cell Receptor