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Modulation of Immune Cell Function by α_1-Adrenergic Receptor Activation

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Abstract

The sympathetic nervous system regulates human immune system functions through epinephrine (Epi) and norepinephrine (NE) activation of adrenergic receptors (AR) expressed on immunocompetent cell populations. The anti-inflammatory effects that are most often attributed to increased sympathetic activity have been shown to occur through β_2- and α_2-AR stimulation. However, dichotomous AR effects on immune system function are becoming increasingly apparent. Reports of α_1-AR expression on immune cell populations have been conflicting due to a lack of specific antibodies or subtype-selective receptor ligands. This has made α_1-AR identification difficult and further characterization of α_1-AR subtype expression limited. Nevertheless, there is some evidence suggesting an induction of α_1-AR expression on immunocompetent cells under certain physiological conditions and disease states. Also, the function of α_1-AR activation to modulate immune responses is just beginning to emerge in the literature. Changes in the secretion of inflammatory mediators as well as increased cell migration and differentiation have been described following α_1-AR stimulation on immunocompetent cells. These observations demonstrate the significance of α_1-AR activity in immune cell biology and emphasize the importance for understanding α_1-AR effects on the immune system.

I. Introduction

The endogenous catecholamines epinephrine (Epi) and norepinephrine (NE) are critical for initiating the “fight or flight” response of the sympathetic nervous system. Epi and NE are released from peripheral neurons and the adrenal medulla in response to physical as well as psychological stress to regulate a number of physiological functions including energy metabolism, cardiovascular homeostasis and thermal adaptation. There are extensive interactions of the central nervous system with the immune system and all immune organs are innervated by post-ganglionic sympathetic fibers. Furthermore, sympathetic nerve terminals are located in the vicinity of immune cells that comprise both the innate and adaptive immune system. Moreover, macrophages have recently been shown to synthesize and release catecholamines *in vivo* (Flierl et al., 2007). Consequently, the close propinquity of catecholamines release to cells of the immune system introduces an opportunity for these endogenous AR agonists to regulate immune cell functions.

AR-mediated sympathetic responses to stress are a result of receptor agonist stimulation caused by the increased release of Epi and NE. The AR family is classified according to type (α_1-, α_2- and β-AR), which can be further characterized into nine distinct receptor subtypes (α_{1A}-, α_{1B}-, α_{1D}; α_{2A}-, α_{2B}-, α_{2C}-; β_1-, β_2- and β_3-AR; see review by (Guimarães and Moura, 2001). All three AR types are expressed in the immune system and like glucocorticoid receptors are considered immunosuppressive when activated by Epi or NE. However, there is a growing body of evidence to suggest that AR activation influences the immune response in a less monochromatic way.

AR activation serves many functions in the immune system including modifying the number or proportion of cells participating in an immune response as well as altering individual immune cell responsiveness (Calcagni and Elenkov, 2006; Bao et al., 2007; Pesic et al., 2009). In addition, a variety of immune cell activities are modulated by AR stimulation including cell proliferation, cytokine production, lytic activity, migration and antibody production (Maestroni, 2000; Seiffert et al., 2002; Pesic et al., 2009; Grisanti et al., 2010). Studies examining the β-AR family are the most extensive, with the “anti-inflammatory” β_2-AR subtypes thought to be the predominant AR expressed in the immune system (Elenkov et al., 2000). However, there is growing evidence to suggest a “pro-inflammatory” function of β-AR activation, which is mediated through the β_1-AR subtype (Grisanti et al., 2010). The α_2-AR family has also been extensively investigated and again is regarded as having anti-inflammatory effects when activated (Elenkov et al., 2000). The α_1-AR family is the least characterized AR in the immune system, which is likely due to conflicting reports of their expression as well as function on immune cells (Ricci et al., 1999; Elenkov et al., 2000; Tayebati et al., 2000).

II. α_1-Adrenergic Receptor Expression in the Immune System

The three characterized α_1-AR subtypes (α_{1A}-, α_{1B}- and α_{1D}-) are differentially expressed in many organs and cells of the immune system. Investigation of α_1-AR expression in immune tissues has relied heavily on RT-PCR analysis. Little information is known about α_1-AR subtype localization at the protein level in the immune system since commercially available antibodies have been shown to be non-selective in wild-type and transgenic animal models (Jensen et al., 2009). Therefore, most studies have been performed utilizing PCR techniques, which is prone to contamination or radioligand binding studies that used non-selective ligands. α_1-AR expression is found in murine hematopoietic stem cell progenitor cells during all stages of development from bone marrow to monocytes/macrophages (Muthu et al., 2007). Murine bone marrow expresses α_{1A}- and α_{1B}-AR mRNA while human bone marrow transcriptionally expresses the α_{1B}- and α_{1D}-AR subtypes (Maestroni et al., 1992; Kavelaars, 2002). High levels of α_{1A}- and α_{1B}-AR mRNA for the are present in the human spleen (Price et al., 1993; Faure et al., 1995), while others have reported the expression for all three α_1-AR subtypes (Kavelaars, 2002).

The majority of studies examining immune cell α_1-AR expression have been performed on peripheral blood mononuclear cell (PMBC) preparations, which include several blood cell types including T cells, B cells, NK cells, monocytes and macrophages. Reported α_1-AR expression on PMBC preparations as well as many of the individual cell populations is contradictory. There are numerous reports documenting an absence of α_1-AR expression using PMBC preparations (Casale and Kaliner, 1984; Kavelaars, 2002). Conversely, others have shown no genomic α_1-AR expression on PMBC preparations under normal culturing conditions, but expression could be induced for all three α_1-AR subtypes following phytohemagglutinin (PHA) or lipopolysaccharide (LPS) stimulation (Rouppe van der Voort et al., 2000). *In situ* hybridization techniques have been used to show that the majority of cells in a PMBC preparation are positive for α_{1B}- and α_{1A}-AR expression with α_{1D}-AR

subtypes found to a lesser extent (Tayebati et al., 2000). Immunocytochemistry analysis in this same study confirmed a majority of PMBCs expressing the mature α_{1B}-AR protein with fewer cells expressing the α_{1A}- and α_{1D}-AR subtypes. In other immune cell types, genomic expression of the α_{1A}-AR subtype has been shown in RNA isolated from rat microglia, the resident macrophage of the brain (Mori et al., 2002). Radioligand binding analysis has described the representative expression of all three AR families on human NK cells (Jetschmann et al., 1997). Immature dendritic cells have been shown to express α_{1B}-AR mRNA, which is lost upon maturation (Maestroni, 2000).

Studies which characterize α_1-AR expression on monocytes have been controversial. The human monocytic cell line, THP-1, has been shown to endogenously express α_{1B}- and α_{1D}-AR mRNA, while genomic α_{1A}-AR expression could be induced following treatment with tissue necrosis factor (TNF)-α or interleukin (IL)-1β (Heijnen et al., 2002). Furthermore, evidence for functional α_1-AR expression on primary monocytes isolated from human blood has been described (Takahashi et al., 2005). Conversely, other reports have documented no detectible α_1-AR mRNA from human monocytes (Rouppe van der Voort et al., 1999). However, this same comprehensive study demonstrated that monocytes cultured in the presence of a glucocorticoid, dexamethasone, or the β_2-AR agonist, terbutaline, resulted in the induced expression of α_{1B}- and α_{1D}-AR mRNA. In addition, upregulation of cAMP-dependent protein kinases using dibutyryl cAMP specifically increased α_{1B}-AR mRNA expression. These authors also utilized immunoblot techniques and radioligand binding analysis to confirm these changes in α_1-AR expression at the protein level.

The thymus, an important organ of the adaptive immune system where T cell maturation and differentiation occurs, has been reported to have low mRNA expression for all three α_1-AR subtypes (Kavelaars, 2002). Further translational analysis using immunohistochemistry techniques confirmed α_1-AR thymus expression, predominantly in the subcapsulary/subtrabecular cortex and cortico-medullary junctions, but rarely in the thymic medulla (Pesic et al., 2009). α_1-AR expression was found in this study primarily on thymic epithelial cells, but also was shown on cluster of differentiation (CD)68^+ cells, a monocyte/macrophage marker. Only a small portion of T cell precursors or thymocytes, showed mature α_1-AR protein expression and of this population, the majority were $CD3^-$ cells with lower expression found on $CD3^{low}$ and $CD3^{high}$ thymocytes. Others have demonstrated that matured thymocytes or lymphocytes, isolated from the blood of healthy human patients express mRNA for all three α_1-AR subtypes, with α_{1B}-AR expression being the highest (Ricci et al., 1999). Radioligand binding analysis in this investigation confirmed mature translational expression on lymphocytes with a calculated [^{3}H]-prazosin affinity (K_d) value of 0.65±0.05 nmol/L for these α_1-ARs with a total receptor density (B_{max}) of 175±20 fmol/10^6 cells.

III. The Role of α_1-Adrenergic Receptors in the Innate Immune System

The human innate immune system is a non-specific means of defense against a pathogenic challenge. This generic means of defense is thought to be a more primitive or non-specific means of protection than the adaptive immune system. Protective systems initiated by innate immune responses include recruitment of immunocompetent cells to sites of infection through production of chemical mediators such as cytokines, activation of the complement cascade to identify and clear invading pathogens, the removal of foreign substances by white blood cells and activation of the adaptive immune system through antigen presentation. Physical barriers such as epithelial cells as well as homeostatic mechanisms such as peristaltic movement, tears and mucus production help to prevent colonization and expedite removal of invading pathogens. Furthermore, innate inflammatory responses create a biological barrier through the release of chemical factors from injured cells, which

establishes an additional obstruction against the spread of infection, while promoting healing by increasing pathogen clearance. Cells of the innate immune system provide the first line of defense against invading pathogens through recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), which initiates cellular and humoral responses. The complement system is a protease C3-convertase cascade, which when activated leads to the recruitment of inflammatory cells while at the same time opsonizing infected cells for destruction through disruption of the plasma membrane resulting in cytolysis.

A. Monocytes

Monocytes are a type of white blood cell responding rapidly to inflammatory signals by moving into the affected tissue and differentiating into macrophages and dendritic cells. Monocytes are involved in phagocytosis, antigen presentation and are major producers of pro-inflammatory cytokines. Reported α_1-AR expression on monocytes has been variable. Using RT-PCR to examine transcriptional expression levels in the human monocytic cell line, THP-1, it was shown that these cells express mRNA for α_{1B}- and α_{1D}-AR subtypes only (Heijnen et al., 2002). Conversely, other reports using primary human monocytes demonstrated no detectable levels for any α_1-AR mRNA (Rouppe van der Voort et al., 1999). There is some evidence suggesting that monocyte α_1-AR expression levels change during certain culturing conditions, possibly explaining the variable literature reports. For example, culturing primary monocytes in the presence of dexamethasone or terbutaline induces α_{1B}- and α_{1D}-AR mRNA expression (Rouppe van der Voort et al., 1999). In other reports, addition of the pro-inflammatory cytokines TNF-α or IL-1β into the media induced genomic α_{1A}-AR expression, while at the same time decreasing α_{1D}-AR subtype expression (Heijnen et al., 2002).

Little evidence is found in the literature as to the function of α_1-AR activation on monocytes. There has been some suggestion that inhibition of α_1-AR signaling on monocytes regulates migration. For example, migration of THP-1 or PMBCs in response to monocyte chemotactic protein-1 (MCP-1) is dose-dependently attenuated by administration of the α_1-AR antagonists doxazosin or phenoxybenzamine (Kintscher et al., 2001). However, this inhibitory effect on the monocyte migratory response was suggested to occur independent of α_1-AR blockade, possibly through enhanced expression of tissue inhibitor of metalloproteinases 1 (TIMP-1). There is also compelling evidence suggesting that α_1-AR stimulation by Epi and NE enhance compliment synthesis. Studies have shown that enhanced complement component 2 (C2) was synthesized from PBMCs treated with increasing concentrations of the selective α_1-AR agonist, phenylephrine (PE), but not when selective β-AR agonist, isoproterenol was used (Lappin and Whaley, 1982). Furthermore, the non-selective α-AR antagonist, phentolamine as well as the selective α_1-AR antagonist, prazosin but not the selective α_2- or β-AR antagonists, yohimbine and propranolol respectively, abrogated the increased C2 synthesis observed in monocytes treated with Epi, NE and PE. Moreover, other complement cascade components including C4, C3, C5, factor B, properdin, C3bINA and β1H were also observed to be increased following PE treatment. Inhibition of monocyte α_1-AR activation also influences expression of signaling components related to T cell activation. Preparations of PBMCs treated with the quinazoline-based α_1-AR antagonists doxazosin, prazosin and terazosin, induced the expression of intercellular adhesion molecule-1 (ICAM-1) and CD40 in a concentration-dependent manner (Takahashi et al., 2005). This study also demonstrated decreased production of the pro-inflammatory cytokine IL-18 from PBMCs using these same receptor antagonists. Alternatively using selective α2-, β1- or β2-AR antagonists did not change levels of ICAM-1, CD40 or IL-18. Recent studies have shown that murine hematopoietic

progenitor cells, ER-MP20$^+$, which are monocyte committed cells, express α_1-ARs on their cell surface that function to increase LPS-mediated TNF-α secretion (Muthu et al., 2007).

Our laboratory has also examined the influence of α_1-AR activation to modulate pro-inflammatory cytokine production from pathogenically challenged human monocytes. Using LPS, which is a component of Gram negative bacterial cell walls and a potent endotoxin, to model inflammation, we investigated changes in the cytokine profile generated from monocytes concurrently treated with PE. An antibody array containing 39 specific antibodies for known mediators of inflammation immobilized on a membrane support (table 1; RayBiotech, Norcross, GA) was used to screen for modulated cytokines/chemokines in response to individual or combined 3 h treatments with 10 μM PE and 25 ng/mL LPS as described previously (fig 1; (Grisanti et al., 2010)). Expression levels of inflammatory mediators from quiescent THP-1 cells were relatively low with the strongest basal expression observed for IL-8 and macrophage inflammatory protein (MIP)-1β (fig 1A). Although a majority of inflammatory mediators did not change for monocytes treated with PE when compared to control, minor qualitative changes in the levels of MIP-1α, MIP-1β and IL-8 were observed (fig 1B). As expected, treatment with LPS qualitatively increased monocyte secretion of many inflammatory mediators including IL-1β, TNF-α, IL-6, the IL-6 receptor, IL-8, IL-10, ICAM-1, TIMP-2 and RANTES when compared to basal levels (fig 1C). Finally, there were characteristic changes in the inflammatory protein expression pattern secreted from monocytes treated concomitantly with PE and LPS (fig 1D). There was a significant qualitative increase in the levels of IL-1β released from PE plus LPS treated monocytes when compared to LPS alone. Conversely, there was a qualitative loss of LPS mediated TNF-α, IL-8 and MIP-1β levels in the presence of PE when compared to monocytes treated with LPS only. These results initially characterize the functional regulation of α_1-AR activation for multiple inflammatory factors from LPS-challenged monocytes and demonstrate the utility of antibody arrays to analyze several mediators of the innate immune response simultaneously.

B. Macrophages

Macrophages are phagocytes residing in tissues functioning as antigen presentation cells to stimulate responses of the adaptive immune system. They also have an important regulatory role in the development of innate immune responses by producing chemical substances including complement proteins, cytokines, chemokines and proteolytic enzymes. There are limited investigations characterizing the expression profile or function of α_1-ARs on macrophages. The previously described ER-MP20$^+$ monocyte committed progenitor cells in mice, which only differentiate into monocytes and macrophages, express functional α_1-ARs that lead to increases in TNF-α secretion through a cooperative mechanism with Toll-like receptor (TLR)4 (Muthu et al., 2007). In the rat thymus, immunohistochemistry techniques co-localized α_1-AR expression with the monocyte/macrophage marker CD68 (Pesic et al., 2009). Functional α_1-ARs were identified on murine RAW264 macrophages when PE and other protein kinase C (PKC) activating agents were used to initiate cell spreading (Petty, 1989). In other studies, PE was also shown to increase primary peritoneal macrophage phagocytosis (Javierre et al., 1975). Kupffer cells are resident macrophages of the liver, which are important for cell-cell communication and are essential for the physiological immune response of the liver. Kupffer cell preparations from control and tumor bearing rats increased production of prostaglandin (PG)E_2 when treated with PE, which could be blocked by the administration of prazosin (Seelaender et al., 1999). Resident macrophages of the brain or microglia in the rat have been shown to transcriptionally express the α_{1A}-AR subtype (Mori et al., 2002) This studies also demonstrated that culturing microglial with LPS and L-serine then treating with PE significantly decreased TNF-α and IL-6 at the transcriptional and translational levels as well as inhibited production of nitric oxide (NO).

PE treatments have also been shown to have an inhibitory effect on LPS-induced NO production in the murine N9 microglia cell line (Chang and Liu, 2000). Osteoclasts are resident bone macrophages that function to resorb old bone formations. Using the receptor activator of nuclear factor *kappa*-light-chain-enhancer of activated B cells (NF-κB) ligand (RANKL) to differentiate murine macrophage RAW264 cells into osteoclasts, investigations have characterized α_{1A}-AR mRNA expression in macrophages with higher levels observed in osteoclasts (Suga et al., 2010). Conversely, transcriptional expression of the α_{1B}-AR subtype was only observed in RAW264 cells and was not found in RANKL differentiated osteoclasts (Suga et al., 2010). This study also examined a postulated α_1-AR-mediated neuro-osteogenic network using primary murine superior cervical ganglia and RANKL differentiated osteoclasts co-cultures. Neurite activation was evoked by treatment with scorpion venom (SV), which subsequently resulted in osteoclast activation as measured by Ca^{2+} mobilization. Treatment of osteoclast alone with SV exhibited no response. Pretreatment of co-cultures with prazosin did not affect SV-mediated neurite activation but did inhibit the osteoclast Ca^{2+} response. Subsequent treatment of RANKL differentiated osteoclasts with PE increased the synthesis of IL-6 validating functional α_1-AR expression on these cells.

C. Dendritic Cells

Dendritic cells (DCs) are important components of the innate immune system, which process and present antigen material to activate cells of the adaptive immune system. Immature murine dendritic cells express α_{1B}-AR subtype mRNA that is lost upon maturation in the lymph nodes (Maestroni, 2000). Correspondingly, migration of immature Langerhans cells, skin DCs, to the lymph nodes in response to NE was inhibited by prazosin, but not by propranolol. Interestingly, pretreatment with yohimbine had the opposite effect by increasing NE-mediated Langerhans cell migration. Conversely, other investigations have identified α_{1A}-AR mRNA expression in murine Langerhans cells as well as in a DC line (Seiffert et al., 2002).

D. Neutrophils

Neutrophils are the most abundant white blood cell in mammals and are vital to the innate immune response. Under normal conditions, neutrophils reside in the blood and upon initiation of inflammation migrate towards the site of injury. An important neutrophil function at the site of injury is to release cytokines, which amplify the inflammatory response. Additionally, neutrophils are phagocytes that ingest and destroy microorganisms or cell debris. Like many cells of the innate immune system literature reports of neutrophil α_1-AR expression is mixed. Early radioligand binding investigations of polymorphonuclear leukocyte (PMN) preparations isolated from human blood, which is a mixed population of cells comprised of eosinophils, basophils and neutrophils, demonstrated no specific binding for [^{3}H]-prazosin (Casale and Kaliner, 1984). While there is little evidence to suggest functional α_1-ARs on neutrophils, α_1-AR activation has a positive effect on the number of circulating cells in the blood or neutrophilia. For example, LPS dramatically increases neutrophilia in rats 3h following injection, while pretreating these animals with reserpine, which depletes catecholamine levels by blocking the vesicular monoamine transporter, significantly decreased blood neutrophilia as a result of LPS (Altenburg et al., 1997). Similarly, pretreatment with phentolamine or prazosin inhibited the LPS-induced increase in blood neutrophil counts, while use of PE alone caused neutrophilia in the absence of LPS. The LPS effects on neutrophilia in rats were not affected by pretreating animals with yohimbine or propranolol.

E. Mast Cells

Mast cells are resident immune cells that contain numerous secretory granules and are best known for their role in allergic and anaphylactic responses. However, mast cells also play an important role in wound healing and host defense mechanisms against pathogens. Activation of mast cells by cross-linking of immunoglobulin (Ig)E receptors or complement proteins, causes cell degranulation, releasing inflammatory mediators into the interstitium. Mature α_1-AR expression has been shown on mast cells from cultured neonatal rat heart cells using immunocytochemistry techniques (Schulze and Fu, 1996). In a murine mast cell line, increasing concentrations of PE or NE treatments could increase ^{14}C-histamine release, which could be blocked by pre-incubation with phentolamine (Moroni et al., 1977). Other investigations have shown a correlation between decreased amounts of the degranulation marker, mast cell peroxidase (MPO) and the protective effects of NE pretreatment on a rat model of heart ischemia-reperfusion injury (Parikh and Singh, 1999). However, prazosin administration during ischemia following NE pretreatment reversed the decrease in MPO observed in ischemic-NE preconditioned animals.

F. Natural Killer Cells

Natural killer (NK) cells are a large, granular type of cytotoxic lymphocyte that plays a major role in rejection of tumors and viral infected cells through the release of cytotoxic granules (Paust et al., 2010). They were named natural killer cells because they do not require any preceding pathogenic stimulation to initiate cell killing. Activation of NK cells by cytokines, Fc portion of antibodies binding to Fc receptors or other activating or inhibiting receptors leads to the release of cytoplasmic granules containing perforin and granzyme causing the target cell to die by apoptosis. Radioligand binding has been performed on $CD16^+$ cells isolated from human blood, which identified α_1-, α_2- and β-AR expression on NK cells (Jetschmann et al., 1997). Expression of α-ARs on NK cells appears to vary depending on external stimuli. For example, infusion of Epi decreased expression of β_2- and α_1- but not α_2-ARs while NE had no effect on AR expression (Jetschmann et al., 1997). Morphine exposure suppresses splenic NK activity, which occurs, in part through α_1-ARs. In lymphocyte populations isolated from mouse spleen, phentolamine administration suppresses morphine-induced NK activity suppression (Carr et al., 1993). Prazosin administration had the same effect on NK activity as phentolamine, while yohimbine showed no change from morphine administration alone.

To circumvent problems with non-specific antibodies and non-selective ligands, our laboratory used α_{1A}-AR-enhanced green fluorescent protein (EGFP) tagged transgenic mice to assess lymphocyte populations (Papay et al., 2006). These mice are under the control of the endogenous promoter and therefore, express the α_{1A}-AR subtype in all naturally occurring cell types throughout the body (Rorabaugh et al., 2005). We focused our investigations on the liver because of the bright green cells present in the sinusoids, the key role in systemic innate immunity regulated by the liver, and that this organ harbors a large population of innate immune cells.

DX5 antibody recognizes the CD49b antigen (Arase et al., 2001) that is expressed on the vast majority of mouse NK cells as well as on 5% of $CD8^+$ cytotoxic T cells (Kambayashi et al., 2001). $DX5^+$ NK cells also display an increased cytotoxicity and indicate that functional subsets exist among NK cell population (Arase et al., 2001). B (bone marrow-derived) lymphocytes (cells) not only play a pivotal role in humoral immunity through the production of antibodies, but also are involved in antigen presentation and regulation of T-cell function (LeBien and Tedder, 2008). CD19 is a specific B cell marker and is present on the earliest B lineage cells during development. CD3 is a general marker for most T cells as it is part of the T cell receptor (TCR) complex present on adult T cells. Both T and B cells also possess

the ability to remember encounter antigens in the form of memory cells, which mediate adaptive immunity (*i.e.*, the subsequent immune response to an encounter is different than the first).

We found that the α_{1A}-AR subtype were not expressed in $CD3^+$ T cells (fig 2A), but instead was expressed in $CD19^+$ B cells (fig 2B) and in $DX5^+$ NK cells (fig 2C). As expected the α_{1A}-AR subtype was also highly expressed in liver vasculature (fig 2D). Hepatic NK cells are located in the sinusoids as indicated in figure 2C and high levels are present in the liver, more than any other organ (Nemeth et al., 2009). Since the liver is a target organ for the metastasis of many cancers and for innate immunity, high levels of NK cells may have an effective anti-tumor effect (Subleski et al., 2006). Localization of the α_{1A}-AR in liver vasculature and immune cells may also account for the high level of this subtype expression in liver membrane preparations analyzed by ligand binding (Rorabaugh et al., 2005) even though the α_{1B}-AR subtype is dominant in the rodent liver (Yang et al., 1998). B cells are of low abundance in the liver comprising less than 10% of the lymphocyte population (Nemeth et al., 2009). However, recent evidence suggests that B cell lymphopoiesis occurs in the liver sinusoids by endothelial cells and we therefore speculate this may account for the high degree of co-localization in smaller cells expressing the α_{1A}-AR (Wittig et al., 2010).

IV. α_1-Adrenergic Receptor Influences on the Adaptive Immune System

The highly specialized adaptive immune system allows the host to recognize and remember specific pathogens so that a strong attack can be mounted every time the pathogen is encountered. The system is highly pliant allowing a small number of host genes to generate huge numbers of diverse antigen receptors uniquely expressed on individual lymphocytes. The adaptive immune system functions to recognize specific non-self antigens to generate maximally effective responses tailored to eliminate specific pathogens or pathogen infected cells. Through this initial response the adaptive immune system also develops immunological memory by forming unique antibodies so that memory cells can be called upon to quickly eliminate the pathogen upon subsequent infections. Lymphocytes are the effector cells of the adaptive immune system of which there are two main types, B cells and T cells. Mature cells that have left the bone marrow or thymus and entered into the lymphatic system are naïve and have yet to encounter their cognate antigen. Upon activation by the B or T cell's cognate antigen, they become effector cells, which are actively involved with eliminating the invading pathogen. Memory cells are long lived lymphocyte survivors of past infections that can recognize specific pathogenic antigens.

A. Peripheral Blood Mononuclear Cells

As stated previously, the majority of studies examining immune cell α_1-AR expression have been performed on PMBC preparations, which includes a variety of blood cell types. In studies that induced PMBC α_1-AR expression using PHA or LPS, subsequent addition of NE increased extracellular signal-regulated kinase (ERK) activation, which could be blocked by pretreating with the irreversible α_1-AR antagonist benextramine, but not yohimbine (Rouppe van der Voort et al., 2000). The functional outcome of NE-initiated ERK activation was not further characterized in this investigation. PE treatment of PMBCs isolated from patients with juvenile rheumatoid arthritis induced the mature expression of IL-6, which is commonly secreted by T cells, when compared to preparations from healthy individuals that showed little change in cytokine levels (Heijnen et al., 1996).

B. T Lymphocytes

T (thymus-derived) lymphocytes or T cells are a type of white blood cell which plays an important role in the adaptive immune response. As stated previously, expression of the

TCR complex is a marker that distinguished T cells from other lymphocytes. There are also several types of mature T cells including T helper cells (T_H cells), cytotoxic T cells (T_C cells), memory T cells, regulatory T cells (T_{reg} cells), natural killer T cells (NKT cells) and δγ T cells (Chaplin, 2010). T_H cells are $CD4^+$ cells that aid other immunocompetent cells in their function such as maturation of B cells as well as activation of T_C cells and macrophages. Activation of macrophage and T_C cells occurs through the presentation of peptide antigens by major histocompatibility complex (MHC) class II molecules on the surface of T_H and other antigen presenting cells. Activation results in rapid cell division as well as cytokine secretion which facilitate a variety of immune responses. T_C cells are $CD8^+$ cells that destroy tumor and viral infected cells through TCRs that recognize specific antigenic peptides bound to MHC class I and CD8 glycoproteins. Memory T cells are antigen-specific $CD4^+$ or $CD8^+$ cells that remain following termination of the infection, which can quickly expand into effector T cells (T_H or T_C) following re-exposure to their cognate antigen. T_{reg} cells are crucial for immunological tolerance by functioning to end T cell-mediated immunity following an immune reaction as well as suppressing auto-reactive T cells that may escape the negative selection process in the thymus. NKT cells are unique in that they bridge the innate and adaptive immune responses. Unlike most T cells, which recognize peptide antigens presented by MHC molecules, NKT cells recognize glycolipid antigens presented by CD1d. Following activation, NKT cells have similar functions as T_H and T_C cells in that they contribute to both cytokine production and release of cytolytic molecules. δγ T cells are a small T cell subset primarily found in the gut mucosa having a distinct TCR made from one δ- and one γ-chain glycolipid.

While α_1-AR expression is not commonly found on T lymphocytes under normal conditions, some investigations suggest α_1-AR expression may be regulated in certain lymphoid compartments or under certain pathologic conditions. For example, lymphocytes from rat mesenteric lymph nodes have been shown to transcriptionally express the α_1-AR (Bao et al., 2007). Activation of these lymphocytes with a T cell mitogen, concanavalin A (Con A), increased the quantity of α_1-AR mRNA over resting lymphocytes. However, this study was unable to determine a potential function for these α_1-AR transcripts in that PE had no effect on Con A-induced proliferation or interferon (IFN)-γ and IL-4 production. In other investigations, transcriptional α_{1A}- and α_{1D}-AR subtype expression was also detected in rat lymphocyte populations from the thymus, spleen and peripheral blood (Schauenstein et al., 2000). However, this study documented a decreased in α_{1A}- and α_{1D}-AR mRNA following peripheral blood lymphocyte (PBL) treatment with Con A. These investigators also showed no differences in the mRNA expression of α_{1A}- and α_{1D}-AR subtypes between $CD4^+$ and $CD8^+$ T cells. Radioligand binding studies found a correlation of [^{3}H]-prazosin binding site densities between PBL preparations isolated from spontaneously hypertensive rats (SHR) and humans diagnosed with essential hypertension (Veglio et al., 2001). In this investigation, isolated PBL from both human hypertensive and SHR showed a significant decreases in the [^{3}H]-prazosin B_{max} when compared with Wistar-Kyoto (WKY) rat controls or normotensive individuals. Using subtype-selective α_1-AR antagonists to characterize these specific [^{3}H]-prazosin binding sites in humans, the authors described the mature expression for all three α_1-AR subtypes in both hypertensive and normotensive subjects. The α_{1B}-AR subtype was the highest expressed receptor in normal patients, while PBLs isolated from hypertensive individuals showed no change in the α_{1A}-AR subtype density when compared to control. However, there was decreased α_{1B}-AR subtype expression with an increase in the α_{1D}-AR population from hypertensive patients when compared with normotensive individuals.

Early investigations also have demonstrated the role for α_1-AR activation to inhibit proliferative T cell responses (Heilig et al., 1993). In this study, [^{3}H]-thymidine incorporation of primary murine lymphocytes isolated from immunized animals was

decreased with increasing concentrations of PE, which could be blocked with phentolamine. A more recent comprehensive study using flow cytometry has shown that 11.3% of isolated rat thymus cells express the α_1-AR (Pesic et al., 2009). This α_1-AR expressing cell population primarily consisted of the least mature $CD3^-$ (51.2%) and $CD3^{low}$ (33.2%) to the most mature $CD3^{high}$ (14.2%) differentiating and proliferating thymocytes prior to leaving the thymus. Chronic treatment with the selective α_1-AR antagonist, urapidil, increased the absolute and relative thymic weight in these animals. This result correlated with both absolute and relative thymocyte numbers resulting from a decrease in cell apoptosis. There was also a greater frequency of the nuclear cell proliferation-associated antigen, Ki-67, on thymocytes after urapidil treatment, which is another indication of the negative regulatory function of α_1-AR activation in T lymphocytes.

The overall number of progenitor $TCR\alpha\beta^-$ thymocytes, which give rise to the distinct CD4 or CD8 functional T cell subsets, was likewise increased in urapidil-treated rats (Pesic et al., 2009). In this group the number of $CD4^-CD8^+$ single-positive (SP) and $CD4^-CD8^-$ double-negative (DN) cells remained unaltered. However, a rise in $CD4^+CD8^+$ double-positive (DP) and $CD4^+CD8^-$SP cell subsets was the reason for an increased number of urapidil-treated $TCR\alpha\beta^-$ thymocytes. Urapidil treatment also increased the number of immature $TCR\alpha\beta^{low}$ thymocytes undergoing the selection process. In this group, absolute numbers of $CD4^+CD8^+$DP and $CD4^+CD8^-$SP thymocyte subsets were increased while the cellularity of other subsets remained unaltered. Similarly, urapidil treatment increased the overall frequency of the most mature, post-selected $TCR\alpha\beta^{high}$ cells, which is reflected as large increases in the $CD4^+CD8^-$SP subset, a decrease in $CD4^-CD8^-$DN thymocytes, while the $CD4^-CD8^+$SP and $CD4^+CD8^+$DP populations remained the same.

The Ig Thy-1 (CD90) has been shown to modulate TCRαβ signaling and selection thresholds (Hueber et al., 1997). In urapidil treated thymocytes, CD90 expression was increased in all TCRαβ groups examined over control, again supporting a role for the inhibition of T cell proliferation by α_1-AR signaling (Pesic et al., 2009). The impact of urapidil treatment on T_{reg} maturation in the thymus was similarly examined using this animal model. The unique “self-antigen” RT6.1 previously identified on peripheral T lymphocytes was used to distinguish maturing $CD4^+CD25^+$ T_{reg} thymocytes from cells re-entering the thymus (Agus et al., 1991). In urapidil treated animals, the relative and absolute numbers of $CD4^+CD25^+RT6.1^-$ thymocytes was greater when compared to control. Finally, chronic urapidil treatment also increased both the relative and absolute numbers of maturing $CD161^+TCR\alpha\beta^+$ NKT cells relative to control animals. These results together point to an α_1-AR mechanism which negatively regulates maturation of T lymphocytes in the thymus.

C. B Lymphocytes

B cells are the major producers of antibodies that circulate in the blood plasma and lymphatic system. Upon activation, B cells generate antibodies that recognize unique antigens which neutralize specific pathogens. Each B cell expresses a unique B cell receptor (BCR) that recognizes and binds a particular antigen. Upon antigen recognition, B cells differentiate into effector plasma cells. Plasma cells secrete these specific antibodies, which bind the unique pathogenic antigens on cells to initiate the complement cascade as well as targeting these antigenic cells for phagocytes. While there are varied reports of α_1-AR expression on isolated PMBCs, a mixed cell preparation which contains B cells, there are no reports to suggest α_1-AR expression specifically on B cells (Casale and Kaliner, 1984; Rouppe van der Voort et al., 2000; Tayebati et al., 2000).

V. α_1-Adrenergic Receptors in Immune Tissues

A. Spleen

The spleen is an important immune system organ responsible for removing old red blood cells, maintaining a blood reserve, recycling elemental iron, synthesizing antibodies as well as retaining half the body's monocytes, which allows them to move into injured tissues for differentiation into dendritic cells and macrophages. The spleen is richly innervated by the sympathetic nerves, which has an affect its physiology (Felten et al., 1987). Reports of high transcriptional expression for all three α_1-AR subtypes in spleen have been published (Alonso-Llamazares et al., 1995; Kavelaars, 2002). However, the spleen was one of the first tissues described in which translational α_1-AR homogeneity was demonstrated (Han et al., 1987). Subsequent radioligand binding analysis in bovine and guinea pig spleen demonstrated a homogenous α_{1B}-AR subtype population (Buscher et al., 1996). Conversely, no specific [^{3}H]-prazosin binding could be observed in murine strain (HLG) broken cell spleen preparation (Yang et al., 1998).

There is also evidence to suggest functional α_1-AR expression in the spleen. Electrical stimulation (ES) of isolated murine spleen slices inhibits basal IL-6 secretion, which is attenuated by phentolamine (Straub et al., 1997). Application of the α_1-AR agonist methoxamine mimicked the inhibitory response of ES on basal IL-6 levels. In other studies, NE treatment in the presence propranolol enhanced the murine IgM antibody response in primary spleen cells immunized with sheep erythrocytes *in vitro* (Sanders and Munson, 1984). In a subsequent investigation, methoxamine was used to demonstrate that early IgM increases from immunized murine spleen cells was mediated through α_1-AR activation, while late IgM changes observed in the presence of clonidine were facilitated by α_2-AR stimulation (Sanders and Munson, 1985). Additionally, there is evidence linking changes in spleen α_1-AR activation with chronic inflammatory disease states (Straub et al., 2008). In this study, ES of the splenic nerve in an early type II collagen-induced arthritis (CIA) mouse model showed a decrease in IFN-γ secretion compared to control animals, which was partially reversed in the presence of the α_1-AR antagonist benoxathian.

B. Thymus

The thymus is an organ important for T cell maturation and differentiation as well as contributing to the production and secretion of additional factors that influence immune system function. The thymus is comprised by a central medulla and peripheral cortex, which is entirely surrounded by an outer capsule. The peripheral cortex is where thymocyte development begins as well as TCR gene rearrangement and positive T cell selection occurs. Conversely, the medulla is the location of late T cell development where a majority of negative selection happens. There are two main thymus cell types; thymic stromal cells, which include cortical epithelial cells and thymic medullary epithelial cells; and cells of hematopoietic origin such as dendritic cells, thymocytes or T cell precursors. The thymus is innervated by post-ganglionic sympathetic nerve fibers which use NE as the primary neurotransmitter (Felten et al., 1987).

In addition to the previously described expression of α_1-ARs on thymocytes, which are the hematopoietic progenitor T cell precursors (Pesic et al., 2009), mRNA for all three α_1-AR subtypes is reportedly expressed in the human thymus (Kavelaars, 2002). Further analysis using immunohistochemistry delineated the location of α_1-ARs predominantly in the subcapsulary/subtrabeculary cortex and cortico-medullary junction, with rare expression in the thymic medulla (Pesic et al., 2009). Cell specific α_1-AR expression occurs primarily on thymic epithelial cells, but also can be found on CD68$^+$ cells, a monocyte/macrophage marker, located in the outer cortex and cortico-medullary junction (Pesic et al., 2009).

α_1-AR expression in the subcapsulary/subtrabeculary cortex and cortico-medullary junction suggests a role for these receptors in early T cell development and proliferation. For example, previous studies have demonstrated increased lymphopoiesis and greater mitogen reactivity over control from cultured fetal thymus explants incubated in the presence of PE (Singh, 1979). Additionally, α_1-AR blockade using urapidil has been shown to decrease the proportional thymus weight in immature rat pups, which was the result of reduced total thymocyte number (Plećaš-Solarović et al., 2005). This decrease in thymocyte cell number was localized to the cortex, but not observed in the medullary compartment. Chronic urapidil treatment also resulted in a decreased population of $CD4^+CD8^-$SP cells with a concomitant increase in $CD4^-CD8^+$SP cells, supporting the idea that α_1-ARs influence thymocyte proliferation.

In contrast to immature pups, chronic urapidil treatment increased absolute and relative thymic weigh in adult rats (Plećaš-Solarović et al., 2005; Pesic et al., 2009). Both absolute and relative thymocyte numbers were also increased in urapidil-treated rats (Pesic et al., 2009). Using annexin V as an indicator of cell death, this study showed a decreased frequency of annexin V+ thymocytes in urapidil-treated animals when compared with control. Using a marker of nuclear cell proliferation, Ki-67 immunostaining was a significantly greater in the subcapsular/subtrabecular cortex of rats treated with urapidil as compared to control. As described previously, this investigation also demonstrated an overall increase in thymocyte numbers, which resulted in proportional changes of CD4CD8 T cell populations.

C. Blood/Circulating Cytokines

α_1-AR investigations using individual cell populations and isolated tissues provides a detailed account of their expression and potential function. However, the immune system is a complex network of interacting cells and it is therefore important to know how α_1-ARs are affecting the immune response under physiological conditions *in vivo*. For example, prazosin treatment alone has no effect on murine basal plasma IL-1β levels, but prazosin pretreatment could block increases in plasma IL-1β as a result of intraperitoneal LPS injection (Dong et al., 2002). Similarly, another investigation demonstrated decreased TNF-α levels in mice pretreated with prazosin prior to LPS injection when compared to LPS treatment alone (Sugino et al., 2009). Conversely, both studies showed that prazosin pretreatment further increased levels of the anti-inflammatory cytokine IL-10 in LPS treated animals when compared LPS treatment only.

D. Non-Immune Tissue

α_1-ARs in tissues not considered part of the immune system have been shown to influence immune processes in a number of different ways. In transfected α_{1A}-AR rat fibroblasts, oligonucleotide microarray technology demonstrated that following Epi treatment that several mediators of inflammation, cell motility and adhesion were temporally altered, which was confirmed using RT-PCR and immunoblot analysis (Shi et al., 2006). Some notable observations included increased levels of IL-6 following 1 h Epi treatment, followed by a decreased level after 18 h that was still significantly above levels detected from non-stimulated control cells. Likewise, levels of the neutrophil chemoattractant CXC chemokine, Gro (CXCL1) were increased after 1 h Epi treatment, but alternatively dropped to levels significantly below basal after 18 h. No change was noted after 1 h for the T cell proliferation cytokine IL-15 and the inflammatory transcriptional regulator, high mobility group box 2 (Hmgb2) protein when compared to control, but after 18 h transcriptional levels for these targets were significantly decreased and increased, respectively. Similarly, hyaluronan synthase 2 that synthesizes a component of the extracellular matrix hyaluronan and the hyaluronan receptor (CD44), which mediates lymphocyte binding showed no change

from basal levels after 1 h, but both were significantly increased after 18 h of Epi treatment. These changes in hyaluronan targets were also observed in treated a rat thoracic aorta smooth muscle cell line (A-10) as well as in DDT1-MF2 hamster smooth muscle cells transfected with the human α_{1A}-AR subtype.

Increased IL-6 levels following α_1-AR activation was also shown in primary murine neonatal cardiomyocytes (Perez et al., 2009). This study further elucidated the molecular mechanisms responsible for the observed increased cytokine levels, which included stabilization of the IL-6 transcript. In addition, unique α_1-AR-mediated signaling pathways were found to be necessary for the increased cardiomyocyte IL-6 translation observed in this study. Specifically, pharmacological inhibition of p38 mitogen-activated protein kinase (MAPK) and NF-κB significantly decreased IL-6 levels when compared to Epi treatment. Transgenic mice over-expressing a constitutively active (CAM) α_{1A}-AR construct under control of the endogenous mouse promoter also showed significantly increased IL-6 serum levels when compared with CAM α_{1B}-AR transgenic or nontransgenic animals (Perez et al., 2009). Remarkably, these CAM α_{1A}-AR mice showed no signs of inflammation and were protected against myocardial ischemia suggesting that increased IL-6 levels may be a beneficial and adaptive α_1-AR cardioprotective mechanism (Rorabaugh et al., 2005).

V. α_1-Adrenergic Receptors in Disease States

Given the summary of information to date, an immunomodulatory role under pathophysiological conditions can be brought forward for α_1-ARs expressed in the immune system. For example, α_1-AR activation has been proposed to be important in the development of experimental autoimmune encephalomyelitis (EAE), an inflammatory demyelinating disease of the central nervous system that is often used as a model for multiple sclerosis (Brosnan et al., 1985). Development of EAE results from T lymphocyte sensitization against the myelin basic protein (MBP) and is a typical inflammatory delayed-type hypersensitivity response. Rats sensitized to MBP were analyzed histologically for assessment of inflammatory cell infiltration as well as clinically (*e.g.*, muscle weakness, ataxia, impaired respiration) using a scaled index to grade EAE development. In MBP sensitized male rats, maximal signs of the disease were observed at 13 days post-inoculation (dpi) with a peak clinical index score of 3.9. Conversely, in sensitized prazosin treated animals, there was a dose-dependent increase when peak clinical signs were observed (15 dpi) as well as a decreased peak clinical index (2.2). These observations were specific for α_1-AR antagonism because treatment of MBP sensitized rats with yohimbine or propranolol had the opposite effect by increasing and extending the signs and duration of the disease. This study also described a dose-dependent decrease of brain and spinal cord immune cell infiltration from prazosin treated animals when compared with control. Other investigations demonstrated changes in the permeability of the blood-brain barrier following induction of EAE (Goldmuntz et al., 1986). In this study, immune cell infiltration occurred less rapidly in prazosin treated animals, with no differences from control in cell infiltration at the disease peak. As a result, it is unclear if prazosin effects EAE disease progression is the result of vascular changes or alterations in immune cells function.

There are several studies correlating α_1-AR expression with disease onset or severity. For instance, PMBC preparations are most often reported to have little to no α1-AR expression. However, PMBCs isolated from patients with juvenile rheumatoid arthritis have increased IL-6 production following PE treatment, which was abolished in the presence of doxazosin (Heijnen et al., 1996). Conversely, PE treatment of PMBCs isolated from normal patients demonstrated a decrease in the generation of IL-6. Using a CIA animal model of autoimmune disease, other investigations have focused around the sympathetic nervous systems effect on splenic function in relation to INF-γ secretion (Straub et al., 2008). In this

study, ES was used to release NE from splenic sympathetic nerve terminals in an isolated perfused slice preparation. ES significantly decreased basal IFN-γ levels in CIA mice, which was partially reversed in the presence of benoxathian, indicating that NE acts on α_1-ARs to inhibit INF-γ secretion. In parallel experiments, T cell depletion with anti-CD3 antibodies completely eliminated the basal response from CIA spleens, indicating INF-γ secretion is a T cell-dependent process.

One of the most common causes of intensive care unit patient death is shock due to sepsis, in which cytokine overproduction by the immune system results in systemic vasodilation and circulatory failure due to decreased vasoconstrictor reactivity (Russell, 2006). Patients diagnosed with sepsis require increasing doses of NE in order to maintain blood pressure via α_1-AR activation on vascular smooth muscle cells. In a rat model of LPS-induced endotoxemia, increased levels of TNF-α and IL-1β were associated with decreased mRNA levels for all three α_1-AR subtypes (Bucher et al., 2003). Addition of TNF-α and IL-1β to rat renal cells decreased levels of α_{1B}-AR subtype expression as assessed by [^{3}H]-prazosin binding. In other studies, blocking vasculature α_1-ARs using prazosin had the same cardiovascular effects on control mice as observed in cecal ligation and puncture (CLP)-induced septic mice (Schmidt et al., 2009). Using dexamethasone treatment or RNA interference technology to decrease levels of cytokine expression caused an attenuated cardiovascular effect and α_1-AR downregulation in CLP mice when compared to control. Small interfering RNA treatment specific for NF-κB also prevented downregulation of α_1-ARs and inhibited cardiovascular dysfunction of CLP mice in this study.

VI. Conclusions

α_1-AR expression on various immunocompetent cell populations has been reported and has been shown to be regulated during pathophysiological processes. However, α_1-AR function to modulate immune cell responses is just beginning to be understood. α_1-AR activation appears to alter production of inflammatory mediators from certain cell types including monocytes, macrophages and myocytes. Additionally, α_1-AR signaling plays a role in dendritic cell migration, lymphopoiesis and mast cell degranulation. A better understanding of how α_1-ARs regulate immune system function could uncover potential therapeutic strategies for modulating pathophysiological responses in human diseases where chronic, inappropriate or hyperactive inflammation is an underlying etiology.

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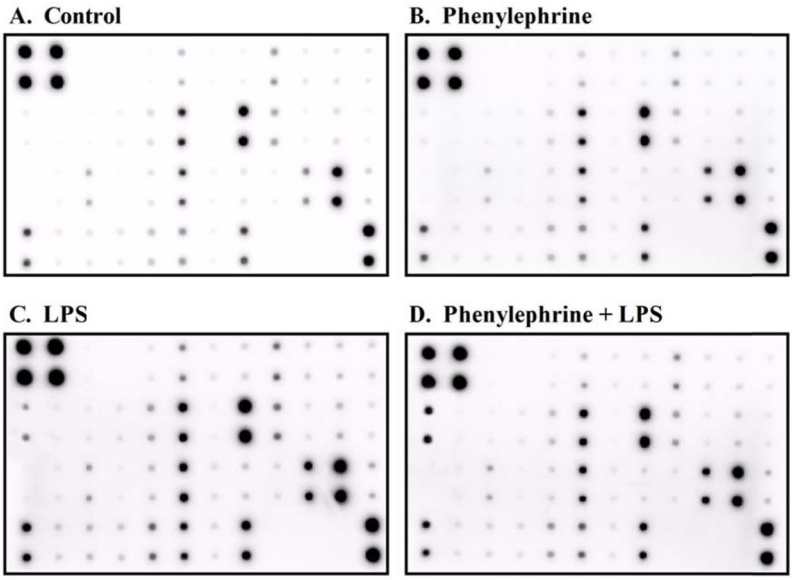

Figure 1.
Representative antibody array membrane for specific inflammatory cytokines (see Table 1 for layout) incubated with conditioned media taken from THP-1 cells treated with (A) culture media, (B) 10 μM PE, (C) 25 ng/mL LPS or (D) 10 μM PE plus 25 ng/mL LPS.

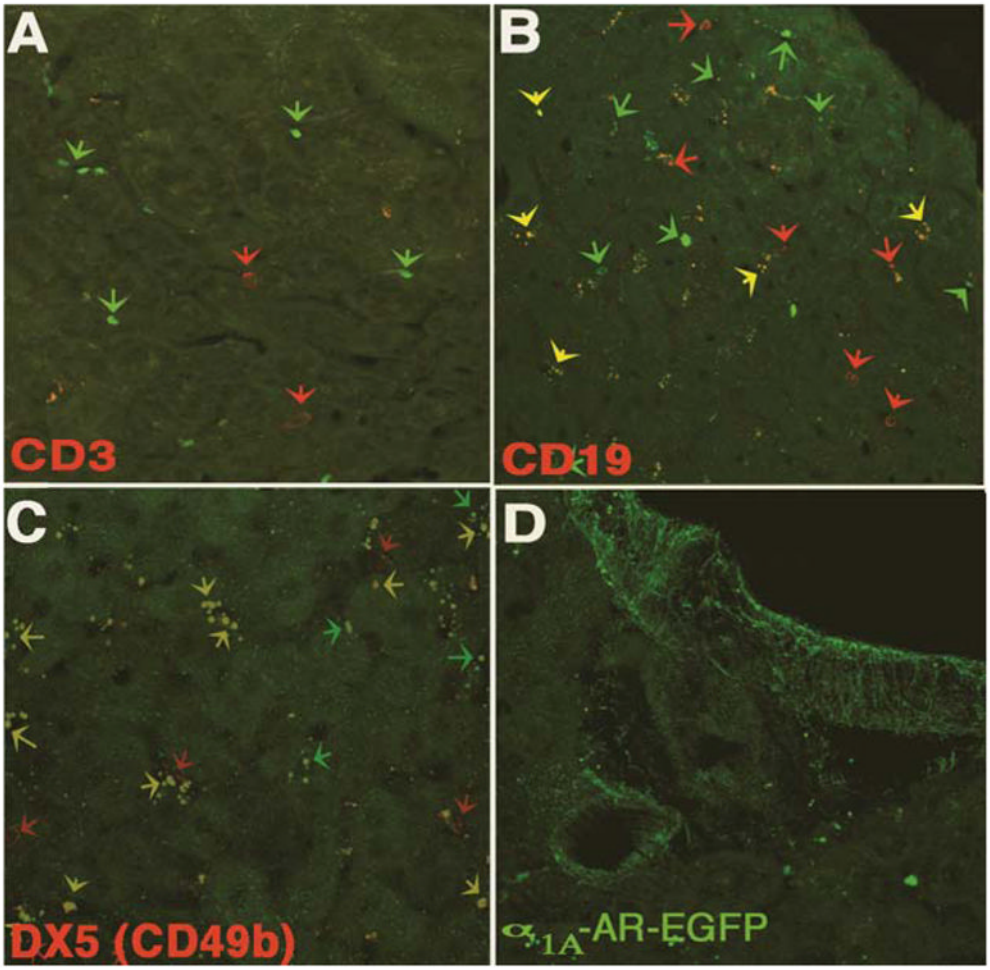

Figure 2.
Immunohistochemistry of α_{1A}-AR-EGFP liver tissue sections reacted with (A) CD3 antibody (T cells), (B) CD19 antibody (B cells), or (C) DX5 antibody (NK cells). (D) Designates α_{1A}-AR-EGFP expression in the liver vasculature.

TABLE 1

Map of Cytokine/Chemokine Antibody Array

POS	POS	NEG	NEG	EOTAXIN	EOTAXIN-2	GCSF	GM-CSF	ICAM-1	IFN-γ	I-309	IL-1α
POS	POS	NEG	NEG	EOTAXIN	EOTAXIN-2	GCSF	GM-CSF	ICAM-1	IFN-γ	I-309	IL-1α
IL-1β	IL-2	IL-3	IL-4	IL-6	IL-6Sr	IL-7	IL-8	IL-10	IL-11	IL-12 p40	IL-12 p70
IL-1β	IL-2	IL-3	IL-4	IL-6	IL-6Sr	IL-7	IL-8	IL-10	IL-11	IL-12 p40	IL-12 p70
IL-13	IL-15	IL-16	IL-17	IP-10	MCP-1	MCP-2	M-CSF	MIG	MIP-1α	MIP-1β	MIP-1δ
IL-13	IL-15	IL-16	IL-17	IP-10	MCP-1	MCP-2	M-CSF	MIG	MIP-1α	MIP-1β	MIP-1δ
RANTES	TGF-β1	TNF-α	TNF-β	s TNF RI	s TNF RII	PDGR-BB	TIMP-2	BLANK	BLANK	NEG	POS
RANTES	TGF-β1	TNF-α	TNF-β	s TNF RI	s TNF RII	PDGR-BB	TIMP-2	BLANK	BLANK	NEG	POS

Positions on membrane support of antibodies that recognize specific mediators of inflammation.

Abbreviations used are **POS**, positive biotinylated protein control; **NEG**, negative BSA control; **GCSF**, granulocyte-colony stimulating factor; **GM-CSF**, granulocyte-macrophage colony stimulating factor; **ICAM**, intercellular adhesion molecule; **IFN**, interferon; **IL**, interleukin; **IL-6Sr**, interleukin-6 soluble receptor; **IL-12 p40**, interleukin-12 p40 subunit; **IL-12 p70**, interleukin-12 p70 subunit; **IP-10**, IFN receptor inducible protein 10; **MCP**, monocyte chemoattractant protein; **M-CSF**, macrophage-colony stimulating factor, **MIG**, monokine induced by γ-interferon; **MIP**, macrophage inflammatory protein; **TGF**, tissue growth factor; **TNF**, tissue necrosis factor; **s TNF R**, soluble tumor necrosis factor receptor; **PDGR**, platelet-derived growth factor; **TIMP**, tissue inhibitor of metalloproteinases.