Human T and Natural Killer Cells Possess a Functional Renin-Angiotensin System: Further Mechanisms of Angiotensin II–Induced Inflammation

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The renin-angiotensin system (RAS) plays an important role in the regulation of inflammation and in the progression of chronic kidney disease. Accumulation of inflammatory cells into the renal parenchyma has been a hallmark of chronic kidney disease; however, little is known concerning the presence and the function of RAS elements in T and natural killer (NK) cells. Here is reported a co-stimulatory effect of angiotensin II (AngII) by showing an augmentation of mitogen and anti–CD3-stimulated T and NK cell proliferation with AngII treatment. Angiotensinogen and AngI also generated the same effect, suggesting that NK and T cells have functional renin and angiotensin-converting enzyme activity. Indeed, they express renin, the renin receptor, angiotensinogen, and angiotensin-converting enzyme by mRNA analysis. Flow cytometric analysis and Western blot revealed angiotensin receptor 2 (AT_2) expression in T and NK cells, whereas AT_1 expression was found in T and NK cells and monocytes by Western blot. These receptors were shown to be functional in calcium signaling, chemotaxis, and proliferation. However, AT_1 and AT_2 antagonists alone or in combination were unable to abrogate completely the effects of AngII, suggesting that another AngII receptor may also be functional in leukocytes. This is the first study to show that T and NK cells are fully equipped with RAS elements and are potentially capable of producing and delivering AngII to sites of inflammation. Because their chemotaxis is enhanced by AngII, this creates a potential inflammatory amplification system.

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Because of its hemodynamic effects, angiotensin II (AngII) plays a central role in the progression of chronic kidney diseases (CKD) and ischemic heart disease (1,2). AngII has been shown to be a potent proinflammatory molecule, and the beneficial effects of renin-angiotensin system (RAS) blockade are due not only to lowering BP but also to a reduction in inflammation (3). One of the main features of CKD is the accumulation of inflammatory cells, which plays a crucial role in disease progression (4), and recruitment of macrophages to the kidney through AngII infusion has been reported in various rodent models (5,6). In both diabetic nephropathy and atherosclerosis, monocytes/macrophages have been reported to play a key role (7–9). Monocytes have also been the primary focus of studies that have examined the interaction of AngII and inflammatory cells (10). However, the importance of T, natural killer (NK), and dendritic cells (DC) in inflammation and vascular disease has only recently begun to be appreciated. DC have been shown to present oxidized LDL to T cells, generating autoreactive T cells and promoting arterial injury (11). NK cells participate through the production of proatherogenic cytokines such as IFN-γ (12). Previous studies on AngII-induced inflammation and its role in kidney disease primarily focused on the induction of inflammatory molecules and the paracrine effects of AngII in vascular remodeling and tissue fibrosis (13,14). Despite these studies having examined the impact of AngII on various steps of inflammation, the direct regulatory effect of AngII on the function and cellular localization of specific immune cells remains to be fully explored in humans. The role of a mobile RAS present on the leukocyte is an important avenue of research that will contribute to a better understanding of the factors that control leukocyte recruitment and will potentially identify important therapeutic targets. In this report, we examine the status of the RAS at the cellular level and the effect of AngII on the function of T and NK cells.

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We dedicate this article to the memory of Joan Sechler, who died on July 5, 2006.

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Materials and Methods

Proliferation Assay

Cells were isolated from healthy volunteers and were cultured as described previously (15).

Cytotoxicity Assay

The effect of AngII on NK cell function was tested using a cytotoxicity assay.

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Culture Supernatant Analysis

The Beadlyte Human Multi-Cytokine Beadmaster Kit (Upstate, Lake Placid, NY) was used according to the manufacturer's protocol.

Real-Time PCR Analysis

Total RNA extraction, reverse transcription, and quantitative PCR (QPCR) were performed as described previously (16).

Western Blot

A Western blot was performed using detection antibodies for AngII type 1 (AT_1) and type 2 (AT_2) receptors.

Flow Cytometric Analysis

Experiments were performed with different antibodies to attempt to show effectively AT_1 and AT_2 surface receptor expression.

Calcium Flux

Fluorescence and calcium flux were measured in leukocytes as described previously (17).

Chemotaxis to AngII

Chemotaxis of specific cell subsets to AngII was assessed. Please refer to the online supplementary material for more details of Materials and Methods.

Results

AngII and Its Precursors Augment Mitogenic T and NK Cell Proliferation

To study the mitogenic effects of AngII on human T and NK cells, we used nylon wool–purified cells that were cultured in the presence or absence of AngII. After isolation, cells were on average 87% $CD3^+$ (T cells), 8% $CD56^+$ (NK cells), and 2% $CD11c^+$ (DC) by flow cytometric analysis. In all subjects studied, AngII alone was unable to stimulate cells in the absence of mitogen; however, AngII significantly increased phytohemagglutinin (PHA)-induced cell proliferation in a dosage-dependent manner (Figure 1A). T cell receptor (TCR)-specific proliferation by plate-bound anti-CD3 was also shown to increase in response to AngII, indicating that AngII can provide co-stimulatory signals when the TCR is specifically engaged (Figure 1B). To determine whether RAS enzymatic components are functionally present in our system, we also added AngI and angiotensinogen to mitogen-stimulated lymphocyte cultures; cells that were cultured with PHA in the presence of 10^{-6} mol/L AngI as well as angiotensinogen increased proliferation to a degree similar to AngII (data not shown), suggesting that these cells are able to convert RAS substrates. To determine whether the observed proliferative effect of AngII could be suppressed, we pretreated cells with 10^{-5} mol/L losartan and PD123319, antagonists for the AT_1 and AT_2 receptors, respectively, for 1 h before culturing them as described. Compared with 10^{-6} mol/L AngII treatment, both antagonists individually blocked proliferation significantly in response to AngII (Figure 1C), although the level of proliferation was higher than that of PHA. Interestingly, when both antagonists were added, proliferation was not significantly reduced compared with 10^{-6} mol/L AngII treatment and in fact increased when compared with either antagonist alone. This suggests that although AngII acts *via* both AT_1 and AT_2, AngII may exert its co-stimulatory effect through another AngII receptor. Cells were also labeled with carboxy fluorescein succinimidyl ester (CFSE) and cultured as described to determine which subsets proliferate in response to AngII. As shown in Figure 1, D and E, $CD8^+$ T cells and NK cells showed a statistically significant, although <20%, increase in proliferation in response to AngII.

We also performed a proliferation assay in which purified cells were depleted of NK cells by bead-mediated removal of $CD56^+$ cells. Under these conditions, the proliferative effect of AngII was blocked (Figure 1F), indicating that AngII may exert its function through NK cells. Upon examination of the direct effect of AngII on T cell proliferation by depleting NK and labeling the remaining T cell population with CFSE, as shown in Figure 1G, we show that $CD8^+$ T cell proliferation in response to AngII is substantially diminished in the absence of NK. To address the direct effect of AngII on NK cell function, we assessed the cytotoxicity of NK cells in the presence and absence of AngII. No difference was noted in cell death when NK cells were treated with AngII (data not shown).

AngII Treatment Increases the Ratio of IFN-γ:IL-4 But Not Their Absolute Concentration

To determine whether AngII affects the cytokine immune response in this culture system, we measured signature cytokines in the cell supernatants. A Th1 immune response has been shown to be important in atherogenesis (12). Figure 2, A and B, shows the absolute concentration of IFN-γ and IL-4 after stimulation of nylon wool–purified cells with PHA and AngII at 10^{-6} mol/L. Although at 48 h a trend was observed in response to AngII treatment toward higher IFN-γ and lower IL-4 production, the difference between AngII treatment and PHA stimulation alone did not reach statistical significance, which could be due to considerable donor variability. We therefore calculated the IFN-γ:IL-4 (Figure 2C) for each donor for these two groups as has been done previously (18,19), which demonstrates a significant although modest shift toward greater IFN-γ production and lesser IL-4 production in response to AngII treatment at 48 h as compared with PHA alone. Of note, the ratio of IFN-γ:IL-4 is thought to be more important in bringing about changes in cytokine signaling, as Th1 and Th2 cytokines are mutually inhibitory (18,19). The induction of the inflammatory chemokines CCL2/monocyte chemoattractant protein-1 (MCP-1) and CCL5/RANTES by AngII was also assessed. Whereas no change occurred in CCL2/MCP-1 production (data not shown), RANTES levels increased almost two-fold with angiotensin treatment of T cells at 72 h (Figure 2D); at earlier time points, RANTES levels were too high to measure. This prolonged production of RANTES in response to AngII may serve to recruit cells to sites of inflammation, or RANTES may act *via* the CCR5 receptor to induce proliferation (20).

RAS Components Are Found on T Cells and NK Cells

Because both AngI and angiotensinogen had similar effects as AngII in augmenting PHA-induced proliferation, the presence of functional renin and angiotensin-converting enzyme (ACE) was suggested in T and NK cells. The presence of RAS

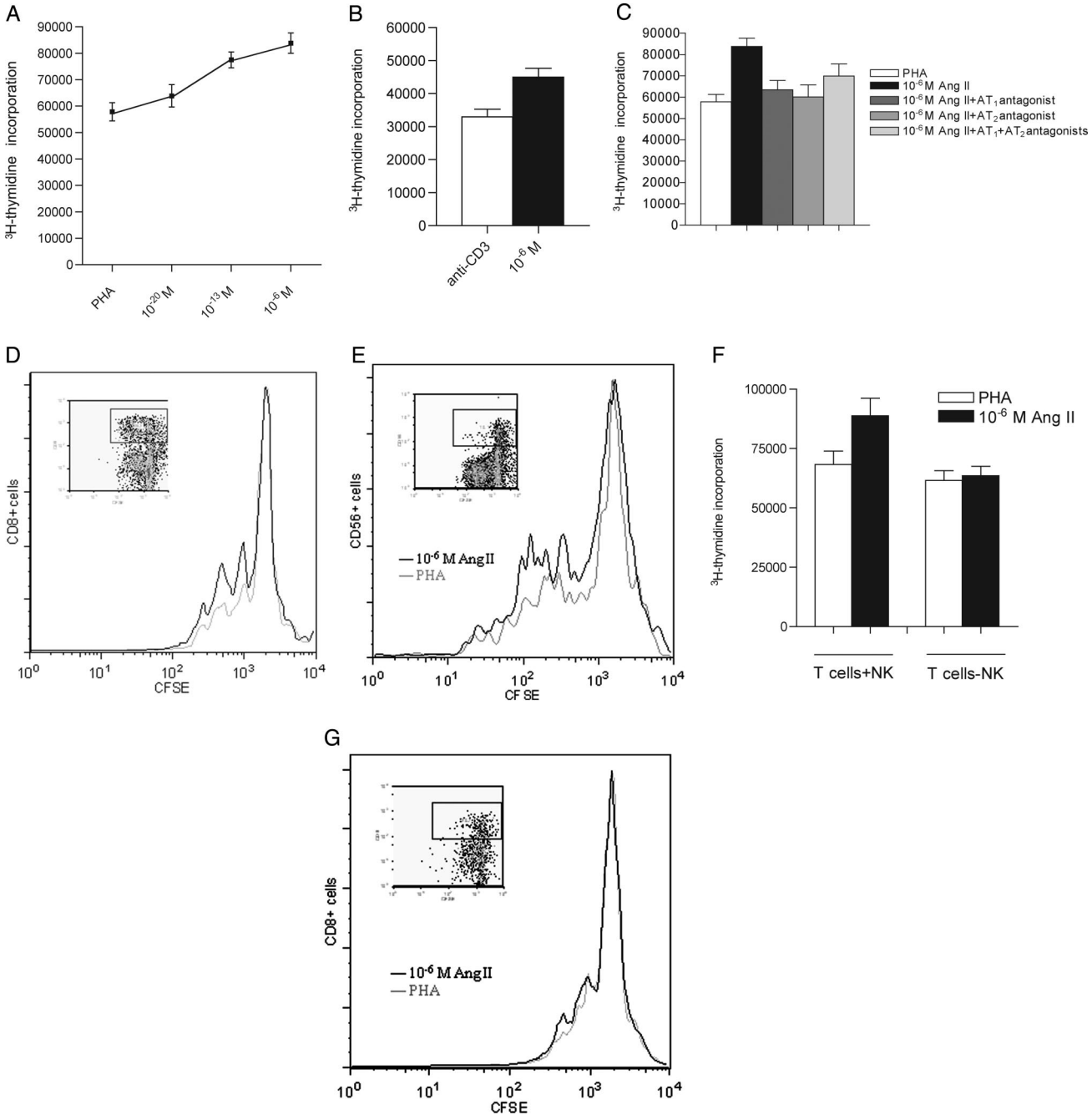

Figure 1. (A) Angiotensin II (AngII) enhances the phytohemagglutinin (PHA)-induced T cell proliferative response in a dosage-dependent manner ($n = 6$ experiments, three donors per experiment [shown as summation of all experiments]; $P < 0.05$ for comparison of 10^{-6} M AngII and PHA alone). (B) AngII also enhances the anti–CD3-induced proliferative response ($n = 4$ experiments, three donors per experiment; $P = 0.001$). (C) In the presence of AngII receptor antagonists, the proliferative effect of AngII is reduced ($n = 4$ experiments, three donors per experiment; $P < 0.05$ for comparison of 10^{-6} M AngII treatment and single antagonist treatment, NS for comparison of 10^{-6} M AngII treatment and treatment with both antagonists). All data are shown as ccpm and are measured by ^{3}H-thymidine incorporation. (D and E) $CD8^{+}$ T cell (D) and natural killer (NK) cell (E) proliferation in response to AngII as measured by carboxy fluorescein succinimidyl ester (CFSE) dilution analysis increased by 18 and 11%, respectively ($n = 3$ experiments, three donors per experiment; $P = 0.0242$ and 0.0123, respectively, in comparison with PHA proliferation). Proliferation is measured as percentage of dividing cells; of note, the increase in dividing cells in response to AngII most often occurred in the first or second division, so that greater precursor division rather than greater number of divisions was evident with AngII treatment. Cells are gated on positively stained cells for CD8 or CD56 (shown in top left corner of histograms), and CFSE dilution with AngII treatment is shown as an overlay to PHA stimulation alone. (F) Compared with T cells in the presence of NK cells (T cells+NK), NK cell depletion (T cell-NK) abolishes the T cell proliferative response to AngII ($n = 3$ experiments, three donors per experiment; $P = 0.03$). (G) when NK cells are depleted, the $CD8^{+}$ T cell proliferation responds much less to AngII as measured by CFSE dilution ($n = 3$ experiments, three donors per experiment; $P = 0.03$).

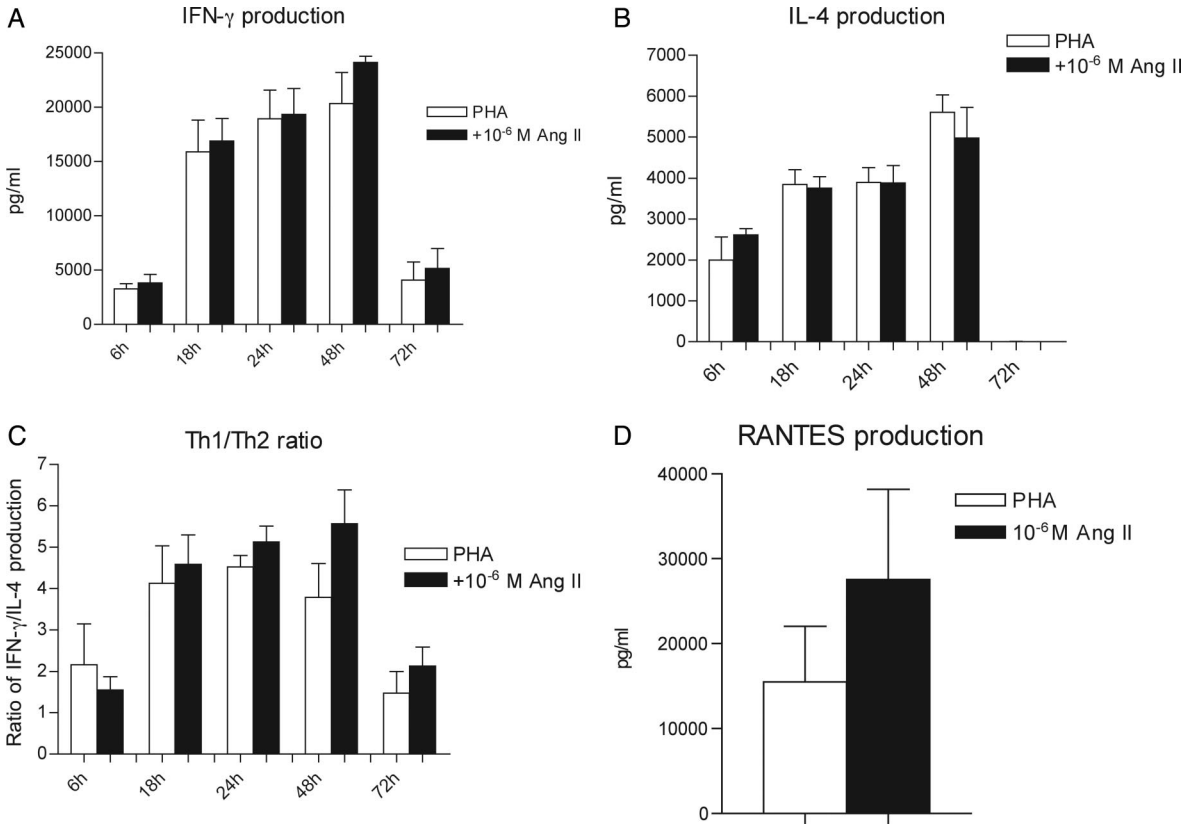

Figure 2. IFN-γ production (A) and IL-4 secretion (B) in response to AngII. Cytokine production is shown in response to PHA alone or in response to PHA and 10^{-6} M AngII treatment. Whereas no difference in absolute concentration of IFN-γ (A) and IL-4 (B) was observed, the ratio of IFN-γ:IL-4 production (C) increased at 48 h ($P = 0.0015$) in response to AngII, demonstrating a shift toward greater IFN-γ production and lesser IL-4 production. Ratios were calculated for each donor for each time point in the PHA alone and AngII-treated groups. (D) Two-fold increase in RANTES production in response to 72 h of AngII treatment ($n = 3$ experiments, three donors per experiment).

elements on T and NK cells has not been comprehensively explored. mRNA was isolated from purified T and NK cells as well as from monocytes and DC, and QPCR was used to quantify the expression of angiotensinogen, ACE, renin, and the renin receptor. Of note, the renin receptor was recently reported to bind both circulatory renin and prorenin, promoting conversion of angiotensinogen to AngI (21). DC were derived from peripheral blood monocytes; their maturity was assessed by studying surface expression for CCR7, HLA-DR, and CD80. T and NK cells both were found to express ACE and the renin receptor, whereas only T cells expressed detectable levels of renin and only NK cells were found to express angiotensinogen (Figure 3). Monocyte and DC RAS expression was previously reported (22,23), and results of these studies are consistent with our data; expression for the renin receptor, however, is novel, and monocytes, immature DC (iDC), and mature DC (mDC) all were determined to express the renin receptor. Expression for renin and its receptor was highest in monocytes, with iDC expressing approximately one tenth the copy number of monocytes (Figure 3, A and B). Monocytes again showed substantially higher expression of ACE (Figure 3D), whereas monocytes and mDC expressed considerable mRNA levels of angiotensinogen (Figure 3C).

AngII Receptor Expression

At the level of the receptors for AngII, few data exist concerning the expression of AT_1 and AT_2 receptors in human T and NK cells. We examined the expression of AT_1 and AT_2 by Western blot and flow cytometric analysis after testing numerous anti-receptor antibodies for specificity. Western blotting demonstrated AT_1 and AT_2 receptor expression in cell lysate protein that was extracted from T and NK cells (Figure 4). Monocytes showed only AT_1 expression by Western blot (Figure 4). The size of the AT_1 protein that was expressed by all subsets was 41 kD, indicating that these cells possess the nonglycosylated form of the receptor; the positive control receptor, however, was glycosylated, resulting in a 50-kD protein. Glycosylation has been shown to increase the functional expression and stability of the AT_1 receptor (24), which could suggest that monocytes, T cells, and NK cells may exhibit low AT_1 surface expression. Indeed, flow cytometric analysis showed negligible AT_1 and slight surface expression for AT_2 on all three subsets compared with isotype control (Figure 5, A through C). T and NK cells showed higher levels of AT_1 expression by Western blot and higher AT_2 expression by FACS. We also assessed the expression of the AT_1 and AT_2 receptors in DC that were cultured from peripheral purified monocytes. Both iDC and

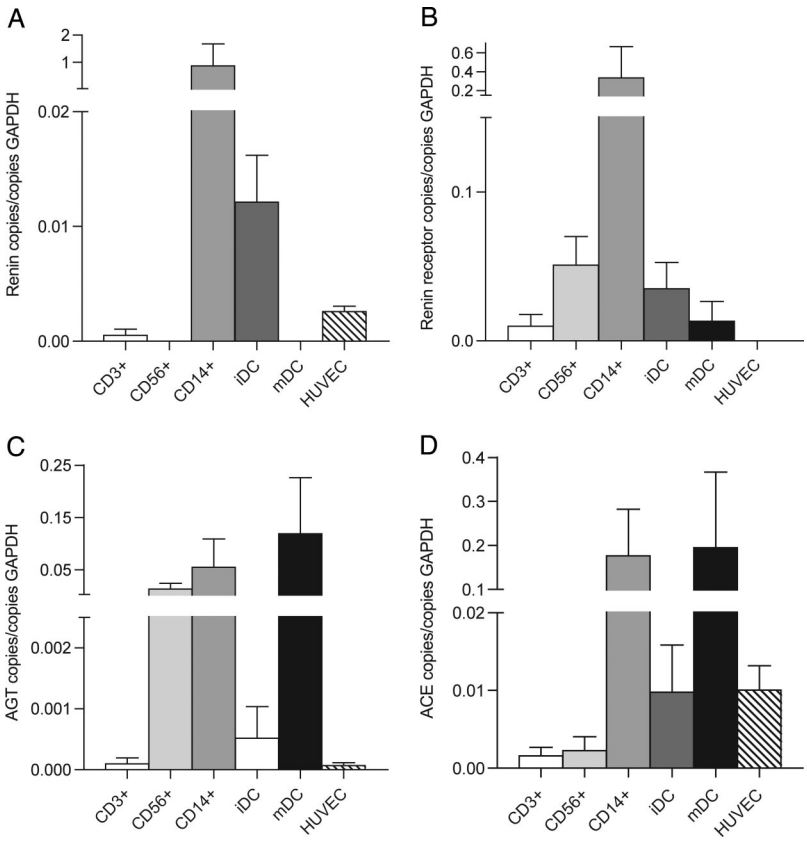

Figure 3. Quantification of mRNA for the renin-angiotensin system (RAS) elements renin (A), the renin receptor (B), angiotensinogen (AGT; C), and angiotensin-converting enzyme (ACE; D). Human umbilical vein endothelial cells (HUVEC) are included as a positive control for RAS components, and mRNA was isolated from cells that were extracted from three to six donors for each subset.

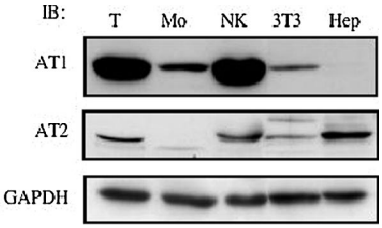

Figure 4. Western blotting detected angiotensin receptor 1 (AT_1) protein expression in $CD3^+$ T cells (T) and $CD56^+$ NK cells (NK) as well as lower expression in $CD14^+$ monocytes (Mo) but at a smaller size than positive control (3T3) protein, presumably as a result of reduced glycosylation. AT_2 receptor was detectable in T cell and NK cell subsets at the expected size but was not detectable in monocytes. Positive control is HepG2 cell lysate (Hep). Membranes were also stripped and reblotted with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to confirm equal loading ($n = 3$ experiments, three donors per experiment).

mDC can be generated *in vitro* as described previously (25). Previous studies that examined AT_1 and AT_2 receptor expression on monocytes and DC were limited by the fact that either a leukemic cell line was used or proper isotype controls were not used (10,26,27). Interestingly, DC, whether immature or mature, showed a substantially higher level of AT_2 receptor expression than all other subsets. DC maturation seemed to decrease AT_2 expression, as iDC had twice that of mDC (Figure 5, D and E). Cells were also permeabilized before flow cytometry for ACE, AT_1, or AT_2 to determine whether these molecules are expressed intracellularly. Little to no difference in ACE and AT_1 expression was found, but AT_2 expression was markedly increased in all subsets after cells were permeabilized (Figure 5, F through H). These data indicate that the AT_2 receptor is predominantly expressed intracellularly and raises the possibility that this receptor, like other chemoattractant G protein–coupled receptors, may be present in storage vesicles that can merge with the cell membrane upon activation (28).

AT_1 and AT_2 in Combination Fail to Block Completely AngII Signal Transduction

To determine whether the angiotensin receptor that we detected on leukocytes was functional, we tested for real-time Ca^{2+} flux in response to AngI and AngII in human peripheral blood mononuclear cells. Activators of phospholipase C, including AngII, release Ca^{2+} from intracellular stores and enhance Ca^{2+} influx. It is interesting that AngI (data not shown) and AngII (Figure 6A) both were able to generate calcium flux at levels comparable to ATP, a potent control stimulus. AT_1 and AT_2 antagonists alone and in combination were able to dimin-

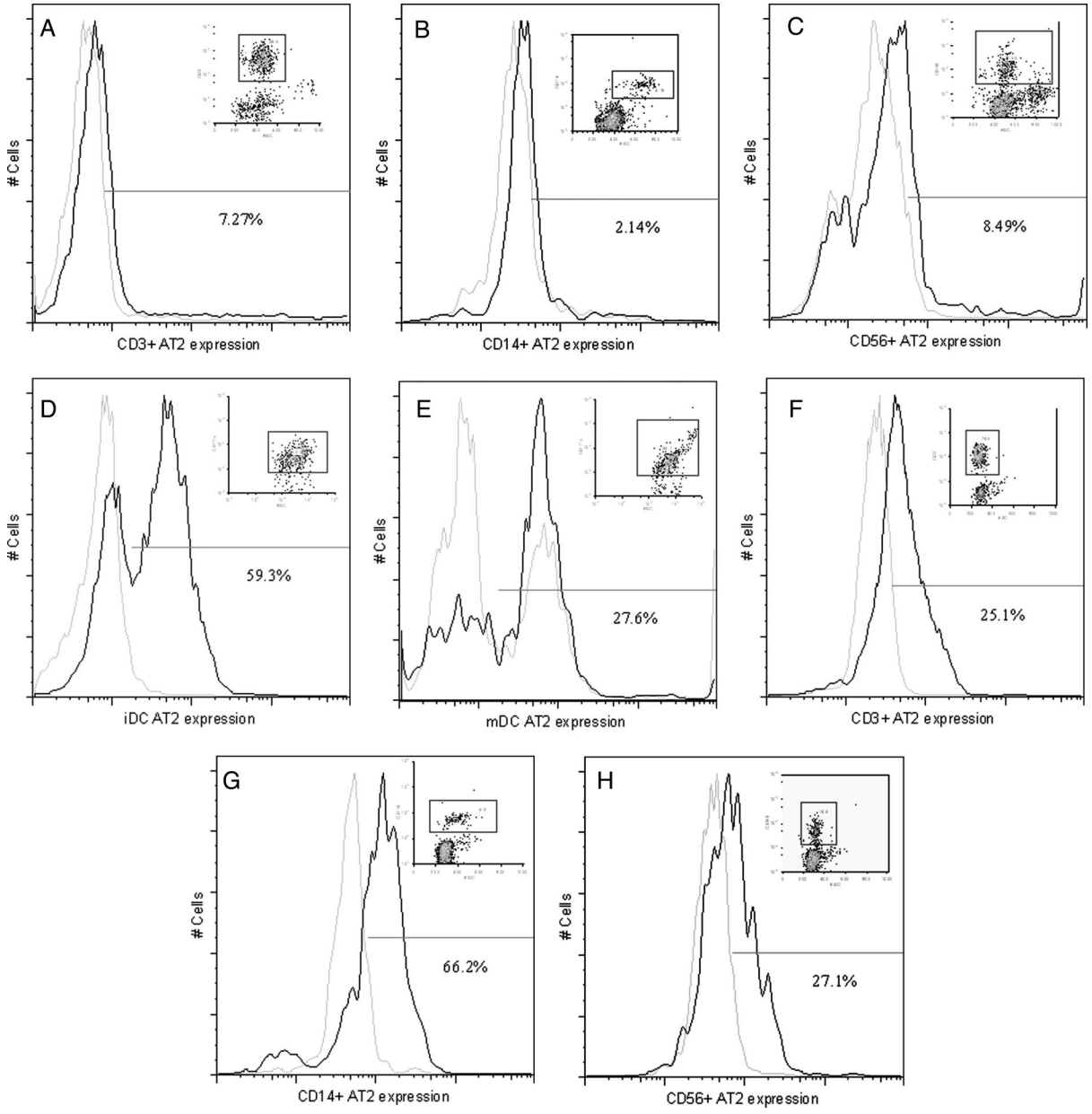

Figure 5. FACS expression of the AT_2 receptor. (A) $CD3^+$ AT_2 surface expression. (B) $CD14^+$ AT_2 surface expression. (C) $CD56^+$ AT_2 surface expression. (D) Immature DC (iDC) AT_2 surface expression. (E) Mature DC (mDC) surface AT_2 expression. Cells are first gated on those that were positive for subset antibodies (shown in the top right corner of each histogram), and data are shown as overlays to the isotype control; percentages are averages of three to five donors. Cells were also permeabilized to determine whether the AT_2 receptor is expressed intracellularly. Intracellular AT_2 expression of $CD3^+$ cells (F), $CD14^+$ cells (G), and $CD56^+$ cells (H) was significantly higher than surface expression.

ish calcium release in response to AngII significantly. The fact that both antagonists simultaneously were unable to suppress completely calcium release again suggests the involvement of another receptor that needs to be identified. Of note, treating cells with AngII receptor antagonists before assessing calcium flux to ATP had no effect, demonstrating that these antagonists do not nonspecifically suppress calcium flux. Leukocyte subset mRNA was also assessed for expression of the AT_4 receptor, a recently identified AngII receptor, and the T and NK cell subsets were found principally to express this receptor (Figure 6B) (29).

T Cells, DC, and NK Cells Migrate to AngII

Recruitment of leukocytes to the site of inflammation is controlled primarily by chemokines. AngII, however, has been shown to have chemotactic properties with respect to monocytes (30), but no data exist on the chemotactic effect of AngII on other human leukocyte subsets; we therefore examined the chemoattractant effect of AngII on purified human T cells, DC, and NK cells. When AngII was added to both the upper and the lower chambers, no chemotaxis occurred, which excluded enhanced chemokinesis. In chemotaxis experiments with isolated T cells, cells migrated 3.5 times more to 10^{-12} mol/L AngII than

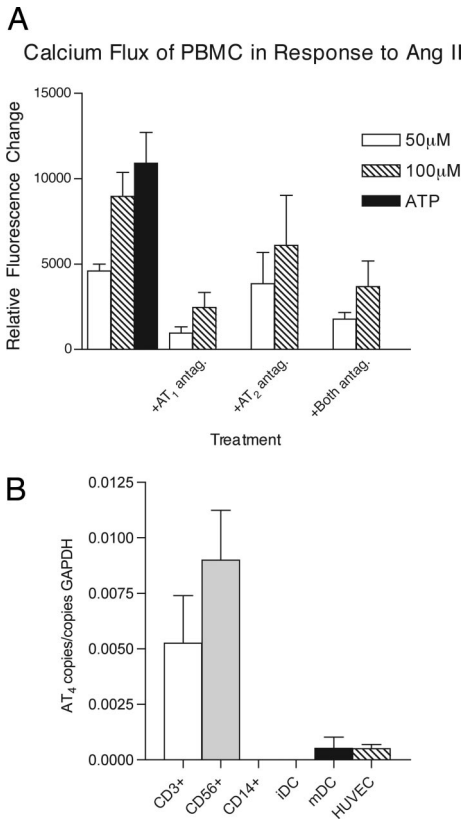

Figure 6. (A) AngII elicits almost the same calcium release from peripheral blood mononuclear cells as the positive control ATP in a dosage-dependent manner. However, combined AT_1 and AT_2 antagonists were unable to block completely the calcium release that was elicited by AngII, suggesting the presence of a third receptor. (B) AT_4 gene expression is demonstrated in T and NK cell mRNA and, to a lesser extent, in mDC and HUVEC mRNA.

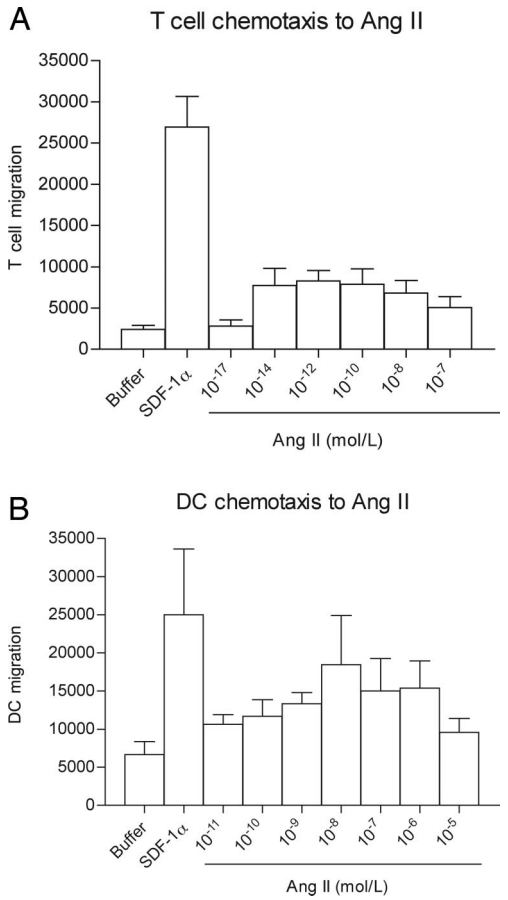

Figure 7. (A) AngII induces T cell chemotaxis. T cells migrate to AngII dosage dependently; at 10^{-12} mol/L AngII, cells migrate nearly 3.5 times as much as buffer ($n = 3$ experiments, two individuals per experiment; $P = 0.0005$). (B) DC migrate potently to AngII ($n = 3$ experiments, two individuals per experiment; $P = 0.05$; at 10^{-9} mol/L AngII).

to buffer ($P = 0.0005$; Figure 7A). The AT_1 and AT_2 antagonists losartan and PD123319 alone or in combination were unable to suppress T cell migration (data not shown), again suggesting the involvement of another angiotensin receptor. DC migrated to 10^{-8} mol/L AngII almost as much as to stromal-derived factor 1α (SDF-1α), a potent chemoattractant, and migrated three times more than to buffer (Figure 7B). Purified NK cells also exhibited directional migration to AngII at 10^{-7} mol/L ($P = 0.002$) and 10^{-8} mol/L ($P = 0.001$; data not shown) than to buffer alone; real-time videomicroscopy with NK cells was performed (see online supplementary movies).

Discussion

The RAS plays a central role in the pathogenesis of cardiovascular and renal diseases. In addition to the hemodynamic-based injuries that are caused by the RAS, a growing body of evidence supports the idea that the RAS exerts its deleterious effects through the induction of inflammatory responses and oxidative stress in the vascular wall. In a model of immune complex nephritis, Ruiz-Ortega *et al.* (4) showed that AngII induces NF-κB activation and CCL2/MCP-1 expression in the renal cortex, an effect that is reduced by ACE inhibition. Others also showed that AngII increases the production of reactive oxygen species, the known activators of NF-κB, and other signal transducers that lead to the activation of proinflammatory cytokine genes (31,32). Because of the importance of T cells, NK cells, and DC in inflammation and in renal and vascular diseases, we explored whether RAS elements are expressed in these subsets, as well as how RAS expression affects their function. One of the first studies to link AngII and the immune system followed studies that showed the co-localization of ACE in alveolar macrophages and in human atherosclerotic plaques that were localized precisely in areas in which macrophages aggregate in atherosclerotic coronary segments (33,34). These data highlight the importance of monocytes/macrophages in the pathogenesis of vasculopathy. A local RAS also was shown to be present in many tissues, indicating that local systems exist and may serve to increase the effects of circulating AngII (29). In humans, the RAS at the cellular level has not yet been comprehensively studied in specific leukocyte subsets. Rat leukocytes have been found to secrete angiotensinogen (35), human neutrophils and mast cells were shown to convert AngI to

AngII (36), and binding sites were found on human mononuclear cells by using radioactively labeled AngII (37). Although the contribution of monocyte-derived macrophages is established in the pathogenesis of kidney and vascular lesions, other leukocytes, such as NK cells, T cells, and DC, are shown to play crucial roles in renal and vascular diseases (16,38,39). AngII was previously shown to augment murine splenic lymphocyte proliferation by Nataraj *et al.* (40) and others (41), and angiotensin receptor blockers suppress murine T cell antigen responses (42). Our data demonstrate the co-stimulatory effect of AngII on human leukocytes; although AngII alone had no effect, NK and T cell proliferation in the presence of mitogen (either PHA or anti-CD3) was potently enhanced when AngII was added. Using CFSE in combination with subtyping antibodies before and after administration of AngII, we demonstrate that $CD8^+$ T cells and $CD56^+$ NK cells proliferate in response to AngII. In agreement with our data, IFN-γ mRNA levels and anti–CD3-stimulated lymphocyte proliferation were shown to be downregulated in renal transplant patients who were on long-term AT_1 blockade (43). In our system, although no difference was evident in the absolute cytokine concentration in response to AngII, stimulating monocyte-depleted NK and T cells with AngII resulted in an increase in IFN-γ:IL-4 at 48 h. A Th1 immune response has been reported to be important in the pathogenesis of inflammation-induced vascular diseases (44). However, the interaction of AngII with Th1/Th2 cytokines and its importance in the development of inflammation remains to be fully explored.

When AngI and angiotensinogen were added to cells, a similar increase in proliferation resulted as in AngII-treated cells, indicating that cells in our system possess the necessary components to convert angiotensinogen to AngI and AngI to AngII. Indeed, we are also demonstrating the presence of RAS elements in human T and NK cells, which shows that they possess a complete enzymatic repertoire to synthesize their own AngII. Renin receptor mRNA was detected in T and NK cells, monocytes, iDC, and mDC. Renin could have cellular effects independent of AngII after binding to the renin receptor (45), and this could create an additional mechanism for renin-induced inflammation.

We also characterized AT_1 and AT_2 receptor expression in T cells, NK cells, and DC. We were able to demonstrate protein expression for both receptors in T and NK cells but found only slight amounts of AT_1 in monocytes by Western blot. Expression of the AT_1 and/or AT_2 receptor was studied by Rasini *et al.* (27) and Nahmod *et al.* (10); however, interpretation of these results is problematic, given that optimal isotype controls were not used for polyclonal antibodies, which are likely to exhibit some nonspecific staining. By FACS, we could not convincingly demonstrate surface expression of AT_1, and even with permeabilization, both AT_1 and the isotype control gave comparable levels of expression. These results are most likely a consequence of the lack of availability of a suitable commercial flow cytometric antibody, given that we were able to detect AT_1 protein by Western blot. Conversely, AT_2 was clearly detectable on the surface of DC and intracellularly in T cells, monocytes, and NK cells. The AT_2 receptor has been thought to antagonize the actions of the AT_1 receptor and therefore to have beneficial effects in hypertension, cell growth, vascular remodeling, and proliferation (46). More recent studies, however, showed that the effects mediated through the AT_2 receptor may in fact complement those mediated through the AT_1 receptor (47,48), which is supported by our data indicating that the AT_2 receptor is expressed on immunocompetent cells that undergo enhanced proliferation, calcium flux, and chemotaxis in response to AngII.

One interesting aspect of AngII in inflammation is its ability to recruit inflammatory cells; recruitment of inflammatory cells is one of the hallmarks of CKD. When AngII is released by inflamed tissue, a gradient is created, which could enhance the directional migration of inflammatory cells. In this regard, human monocytes and murine splenocytes were found to migrate to AngII (30,49). We are reporting for the first time that T cells, NK cells, and DC also migrate significantly to AngII. Given our chemotaxis data, AngII may orchestrate the collective recruitment of leukocyte subsets to sites of inflammation (*e.g.*, atherosclerotic lesions). Our data also show that secretion of the chemokine CCL5/RANTES by the mixture of NK and T cells was upregulated in response to AngII, an effect that previously was noted in rat glomerular epithelial cells (50). Because CCL5/RANTES is known to be chemotactic for cells that bear the CCR1, CCR3, and CCR5 chemokine receptors, this may serve to further recruit leukocytes to sites of inflammation or perhaps to enhance directly T cell proliferation *via* the CCR5 receptor. The calcium flux elicited by AngII showed a comparable response to the calcium flux elicited by ATP. Both antagonists were able to block this receptor signal significantly, indicating the likely presence of both AngII receptors, which is consistent with our QPCR and Western blot data. Furthermore, the fact that the combination of both antagonists was unable to suppress completely calcium flux suggests the presence of another angiotensin receptor in leukocytes, such as AT_4, which may have implications for differences in therapeutic responses.

Conclusion

We have demonstrated that AngII can have co-stimulatory effects on T and NK cells, that specific elements of the RAS exist on T cells, NK cells, and DC, and that DC, NK cells, and T cells migrate potently to AngII. The results of this study will allow us to comprehend better the mechanisms by which AngII leads to inflammation, as RAS antagonists may be used in conjunction with other anti-inflammatory agents as therapy for common diseases in which inflammation plays a major pathogenic role.

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Disclosures

None.

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