Kidney International, Vol. 66 (2004), pp. 2119–2131

PERSPECTIVES IN BASIC SCIENCE

Retinoids in nephrology: Promises and pitfalls

QIHE XU, JAVIER LUCIO-CAZANA, MASANORI KITAMURA, XIONGZHONG RUAN, LEON G. FINE, and JILL T. NORMAN

Department of Medicine, Royal Free and University College Medical School, University College London, London, United Kingdom; Department of Nephrology, General Hospital of Chinese PLA, Beijing, China; Departamento de Fisiologia, Facultad de Medicina, Universidad de Alcala, Alcala de Henares, Madrid, Spain; and Department of Molecular Signaling, Interdisciplinary Graduate School of Medicine and Engineering, Faculty of Medicine, Yamanashi University, Tamaho, Yamanash, Japan

Retinoids in nephrology: Promises and pitfalls.

Background. Retinoids, a family of vitamin A metabolites or analogs, play an important role in regulating cell proliferation, differentiation, and apoptosis.

Methods. The biological importance of retinoids in the kidney and the potential of retinoids in the treatment of renal diseases are reviewed.

Results. Vitamin A deficiency and mutations of retinoid nuclear receptors cause abnormalities in fetal kidneys, which might predispose to adult diseases such as hypertension. Further, the therapeutic value of retinoids in animal models of kidney diseases, such as lupus nephritis, puromycin aminonucleoside nephrosis, anti-glomerular basement membrane nephritis, mesangioproliferative nephritis, and acute renal allograft rejection has been unveiled recently. Retinoids target mesangial cells, podocytes, tubular epithelial cells, interstitial fibroblasts, as well as lymphocytes and macrophages. The anti-inflammation, anti-coagulation effects, and the proliferation- and immunity-modulating actions of retinoids, have been widely appreciated. Our recent in vitro data revealed a direct antifibrotic effect and a cytoprotective effect of retinoids in various renal cell types. In animal studies, the adverse effects of retinoids are generally minimal; however, the clinical use of retinoids in other diseases points to some major side effects. In addition, in vitro, retinoids can induce lipid accumulation in smooth muscle cells and macrophages and increase expression of some proinflammatory molecules, indicating that their clinical toxicity profile in the setting of renal diseases needs to be better understood.

Conclusion. Retinoids not only are important in renal development, but also show promise as a new generation of renal medication and deserve to be tested in clinical trials to clarify their full potential.

Identification of new therapeutic agents has always been an important challenge for nephrologists. Recently, retinoids, a family of vitamin A metabolites or analogs, have been shown to have excellent preventive and therapeutic effects in various experimental kidney diseases, as shown in Table 1 [1–18]. To date, three groups of retinoids have been isolated or synthesized (Table 2) [1–4, 6, 12, 13, 19, 20]. The first group are natural provitamin A, vitamin A, and other retinoic acid (RA) precursors, which cannot bind retinoid nuclear receptors, but are characterized by their potential to be converted into RAs; the second group includes the natural RAs, including all-*trans*-RA (tRA) and 13-*cis*-RA (13cRA), which bind and activate RA receptors (RARs), and 9-*cis*-RA (9cRA), which binds and activates both RARs and retinoid X receptors (RXRs). The third group is composed of synthetic retinoids, which bind one or more RAR or/and RXR isotypes and exert agonistic or antagonistic actions. Some of the third group activate neither RARs nor RXRs, but have selective anti-activator protein-1 (anti-AP-1) activity [2].

Although vitamin A does not bind any of the nuclear receptors, the majority of its functions are retinoid nuclear receptor–dependent; therefore, conversion of vitamin A into RAs that can bind retinoid nuclear receptors is largely a process of vitamin A activation [21] (Fig. 1). The activation of vitamin A occurs via two oxidation steps. It is first converted to retinaldehyde, and then to tRA. Conversion from vitamin A to retinaldehyde is a reversible process that is catalyzed by alcohol dehydrogenases (ADHs) and short-chain dehydrogenases. The irreversible conversion of retinaldehyde to RA is catalyzed by retinaldehyde dehydrogenases (RALDHs), which are the key enzymes of RA synthesis [21]. It is known that tRA is the major biologically active retinoid in both embryonic and adult tissues and that, catalyzed by isomerases, tRA, 13cRA, and 9cRA can be interconverted [22]. The metabolism of RAs is catalyzed by a group of cytochrome P450 (Cyp) enzymes. For example, metabolism of tRA is mainly mediated by Cyp26, which converts tRA to 4-hydroxy-RA and 4-oxo-RA [22]. Although there is compelling evidence to show that 4-hydroxy-RA and 4-oxo-RA are not involved in physiologic retinoid signaling during mouse development [23], at pharmacologic

Key words: retinoids, kidney diseases, fibrosis, inflammation, side effects.

Received for publication March 31, 2004
and in revised form May 25, 2004
Accepted for publication June 18, 2004

Table 1. Effects of retinoids on animal models of renal disease

Model	Response to RAs	References
Acute Thy1.1 nephritis	+	[1, 2]
Chronic Thy1.1 nephritis	+	[3, 4]
Puromycin aminonucleoside nephrosis	+	[5, 6]
Lupus nephritis	+	[7]
Acute kidney allograft rejection	+	[8]
Aging related nephropathy	+	[9, 10]
Unilateral ureteral obstruction	+	[11]
Pyelonephritis	+	[12, 13]
Anti-GBM nephritis (chronic phase)	+	[14]
Anti-GBM nephritis (acute phase)	±	[15]
Radiation nephritis	−	[16–18]

Note: +, renal damage reduced; ±, proteinuria unchanged; −, renal damage enhanced.

concentrations they can activate nuclear receptors and induce cell differentiation [22]. Therefore, the pharmacologic importance of RA metabolites needs to be further defined.

RETINOIDS: MECHANISMS OF ACTION

RAR-, RXR-, and RA response element (RARE)-mediated gene regulation

Biologically active retinoids are characterized by their capacity to bind and activate retinoid nuclear receptors, including RARs and/or RXRs. These receptors are ligand-regulated transcription factors with essential roles in embryonic development and adult physiology, and each has three isotypes (α, β, and γ) [21, 24, 25]. RARs function as a RAR/RXR heterodimer that is activated by RAR agonists. RXR agonists synergize with RAR ligands to activate the heterodimer. In contrast, RXRs function as either a homodimer or a heterodimer with a variety of partners including RAR, peroxisome-proliferator activated receptor (PPAR), thyroid hormone receptor, vitamin D receptor, and orphan nuclear receptors [24].

Agonists and antagonists regulate RAR/RXR heterodimer transcriptional activity by regulating the association of the heterodimer with nuclear corepressors and coactivators, which bind the heterodimer in a mutually exclusive manner [26]. In the absence of RAR agonists, RAR/RXR heterodimers are transcriptionally silenced by binding corepressors. To initiate transcription, the first step is release of corepressors from the heterodimer, which can only be achieved by RAR, but not RXR ligands; the second step is recruitment of coactivators, which is triggered by both RAR and RXR agonists [26]. Upon binding to a RAR agonist, RAR/RXR heterodimers release corepressors, recruit coactivators that confer histone acetyltransferase activity, and initiate transcription of a target gene. RAR and RXR agonists may synergize in activating RAR/RXR heterodimers by collaborating in coactivator recruitment, resulting in increased binding and interaction efficiency of a single coactivator with the heterodimer [26].

The specificity of retinoids in regulating gene expression is at least partially due to specific RAREs in the target genes [24, 25]. There are three different categories of RAREs. The most potent RARE comprises the direct repeat of a consensus sequence, AGGTCA, which is separated by several nucleotides. Less potent RAREs are palindromic repeats, which require over-expression of RARs for activation. Finally, the least responsive RAREs are composed of sequences that are highly degenerate from the consensus, and are scattered randomly along the promoter region. Although most of the effects of tRA and 13cRA are mediated by RAR/RXR heterodimers, endogenously produced RXR activating metabolites can potentially activate other RXR heterodimers or homodimers [24].

By 2002, at least 532 tRA-regulated genes had been described. Of these genes, only 27 have been shown to be directly dependent on RAR/RXR and RAREs, although another 105 appear to be candidates. Data on another 267 genes are ambivalent with regard to direct or indirect regulation by RAR/RXR and RAREs. Most of the remaining 133 genes seem to be regulated indirectly, usually through a transcriptional intermediate [25]. In general, target genes harboring functional RAREs in their regulatory region are immediate-early retinoid response genes that do not require de novo protein synthesis for activation. Retinoid-response genes with no RAREs generally require de novo protein synthesis and thus, show somewhat slower kinetics of expression.

RAR and RXR crosstalk with other transcription factors

Retinoid-activated RAR/RXR heterodimers can positively or negatively interfere with the gene activation mediated by other signalling pathways, including transcription factors AP-1 and nuclear factor κB (NFκB). A number of mechanisms have been implicated in the tRA-induced anti-AP-1 activity of RAR/RXR, including: (*1*) suppression of c-Fos and c-Jun expression [27–29]; (*2*) suppression of the c-Jun N-terminal kinase (JNK) activity in a mitogen activated protein (MAP) kinase phosphatase 1 (MKP-1)–dependent or –independent manner [20, 27–29]; (*3*) disruption of c-Jun/c-Fos dimerization [24]; (*4*) specific suppression of JunB-containing dimers [30]; (*5*) induction of Fra-1, which lacks transcriptional activity [31]; (*6*) suppression of JunD phosphorylation and RNA polymerase II recruitment, and exclusion of CREB-binding protein (CBP) and extracellular-regulated kinases (ERKs) from the AP-1 binding site of the target gene promoter [31]; (*7*) competition with AP-1 for coactivators [24]; and (*8*) induction of the expression of RARβ, which has ligand-independent anti-AP-1 activity [32]. In resident renal

Table 2. Categorization of retinoids

Group	Description	Examples	References
1.	Natural retinoic acid precursors (cannot bind RAR and RXR)	β-carotene, vitamin A (retinol)	[12, 13]
2.	Natural activated retinoids, which bind RAR only	all-*trans*-retinoic acid (tRA)	[1, 6]
		13-*cis*-retinoic acid	[1, 3]
	Natural activated retinoids, which bind RAR and RXR	9-*cis*-retinoic acid	[19]
3.	Synthetic retinoids		
	Agonists		
	Pan-RAR	TTNPB, arotinoid	[2, 4, 20]
	RARα	Am580, AGN195183	[3, 20]
	β	CD2314	[20]
	γ	CD0666, MM11254	[20]
	Pan-RXR	AGN194204, Ro-257386	[2, 4, 20]
	Antagonists		
	Pan-RAR	AGN193109, ER27191	[20]
	RARα	AGN194301, ER50891, CD2503	[20]
	β	LE135, LE550	[20]
	γ	MM11253	[20]
	Pan-RXR	HX531	[20]
	Anti-AP-1 retinoids	BMS-453	[2]

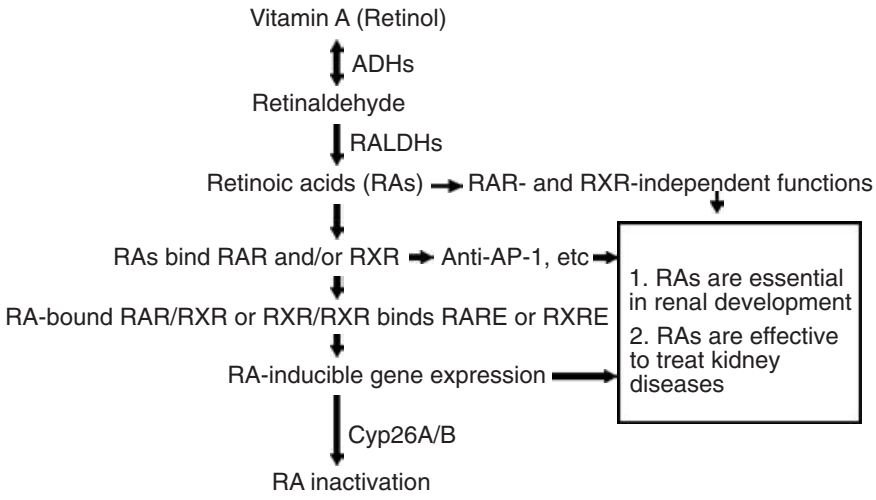

Fig. 1. Biological and pharmacologic functions of natural retinoids. The majority of the functions of vitamin A (retinol) are RA-dependent. Vitamin A does not bind RAR and RXR, but RAs do. The conversion of vitamin A to RAs is catalyzed by ADHs and RALDHs via a reversible oxidation and an irreversible oxidation, respectively. The majority of the functions of RAs are nuclear receptor–dependent by initiation of target gene expression or cross-talk with other transcription factors, such as AP-1. RAs also targets nuclear receptors, kinases, and other protein substrates and, therefore, have RAR- and RXR-independent functions. These mechanisms explain why RAs play important roles in multiple biological and pharmacologic processes, especially during renal development and in kidney diseases. The metabolism and inactivation of RAs is catalyzed by a group of cytochrome P450 (Cyp) enzymes. For example, metabolism of tRA is mainly mediated by Cyp26A and B.

cells, the anti-AP-1 activity of retinoids was recognized as early as 1994 [27], and we recently reported that the anti-AP-1 activity of retinoids is involved in both the prevention of oxidative stress-induced apoptosis [28, 33] and the suppression of proinflammatory molecule monocyte chemoattractant protein-1 (MCP-1) expression in glomerular mesangial cells [34].

The effects of retinoids on NFκB have been shown to be stimulus- or cell type–specific, and both suppressive [35, 36] and stimulatory [37] effects of retinoids on NFκB have been reported. Retinoid nuclear receptors and NFκB were reported to have mutual inhibitory activity in lipopolysaccharide (LPS)-activated macrophages and CV-1 cells via retinoid-dependent RXR-IκBβ interaction [36], inhibition of the NFκB-DNA interactions, and competitive recruitment of transcription integrators [35]. tRA was also reported to significantly reduce nuclear accumulation of NFκB subunits p50 and p65 in mouse mesangial cells, which may account for the inhibition of NFκB-dependent inducible nitric oxide synthase (iNOS) expression by tRA and 13cRA [38]. On the other hand, tumor necrosis factor-α (TNF-α) and 9cRA have been reported to synergistically induce intercellular adhesion molecule-1 (ICAM-1) expression in endothelial cells, via retinoid receptors and NFκB acting in concert at the promoter level in a RARE-dependent manner [37]. Finally, RAR/RXR also interacts with other transcription factors, such as signal transducer and activator of transcription 5 (STAT5), CCAAT/enhancer-binding protein (C/EBP), octamer transcription factor 2A (Oct-2A), and Sp1 [24], although these interactions are less well characterized.

RAR- and RXR-independent roles of retinoids

Although retinoid nuclear receptor knockout results in similar phenotypes to those seen in retinoid-deficient

animals [39], there is emerging evidence to support a role of nuclear receptor–independent mechanisms in the actions of retinoids in a series of biological processes. For example, the biological effects of tRA in tRA-induced cell growth arrest in leukemia cells have been linked to a RAR-independent, mannose 6-phosphate/insulin-like growth factor-II receptor (M6P/IGF2R)-dependent mechanism [40]. In addition, tRA has been identified as a ligand for both RAR-related orphan receptor β (RORβ) and PPARβ/δ. It has been shown that tRA binds RORβ with relatively low affinity (kD = 280 nmol/L) and suppresses its transcriptional activity in a cell type–specific manner [41]. tRA also suppresses the transcriptional activity of RORγ, but not RORα, suggesting that it is a selective ligand for RORβ and γ [41]. PPARβ/δ is an orphan nuclear receptor that has antiapoptotic effects and mediates cell differentiation [42]. It has been reported that tRA binds PPARβ/δ with nanomolar affinity, modulating the conformation of the receptor, promoting its interaction with the coactivator SRC-1, and efficiently activating PPARβ/δ-mediated transcription [42].

Some synthetic retinoids have also been found to have nuclear receptor–independent actions. For example, synthetic retinoids MX781, MX3350-1, and CD2325 induce apoptosis of tumor cells by binding IκB kinase directly, thereby inhibiting the NFκB activation required for cell survival [43]. Notably, although these nuclear receptor–independent, anti-NFκB retinoids can be antagonists or agonists of retinoid receptors, the same mechanism was not observed with natural RAs, indicating that this phenomenon is of pharmacologic, rather than physiologic, significance [43].

Retinoids are also involved in the post-translational modification of proteins by binding covalently to target proteins. tRA is most likely linked to proteins via a thioester bond, a process known as retinoylation or RA acylation, which can occur both in vitro and in vivo [22]. To date, at least 20 retinoylated proteins have been detected in HL60 cells exposed to tRA, and as many as 40 retinoylated proteins have been found in tRA-treated NIH3T3 cells [22]. Like other post-translational modifications of proteins, retinoylation may change the physicochemical properties of the target molecules and induce functional changes. For example, direct binding of retinoids to protein kinase C (PKC) significantly alters PKC function [44].

Retinoid-induced protein kinase activation and suppression

Retinoids have been known to affect the activities of various protein kinases, especially a number of PKC isoforms, and MAP kinases ERK1/2, JNK, and p38. It has been reported that retinoids, including tRA, suppress PKCβ expression [45] and induce PKCα and θ expression and activation [45–47]. Interestingly, a recent in vitro study showed that pharmacologic concentrations of tRA, 9cRA, 4-OH-tRA, and tRA glucuronide directly bind PKCα, hampering binding of phosphatidylserine to PKCα, and preventing PKCα activation [44]. Therefore, the effects of retinoids on PKCα may be dose-dependent, stimulating enzyme activity at physiologic concentrations, and suppressing activity at pharmacologic concentrations. In contrast to PKCα and θ, PKCδ protein expression is not induced by tRA; however, tRA significantly induces the phosphorylation and activation of PKCδ in leukemia and cancer cells [48].

We and others have reported that tRA enhances ERK1/2 phosphorylation [20, 49]. tRA-induced ERK2 activation in HL-60 leukemia cells was reported to be dependent on both RAR and RXR [49]; however, retinoids may also induce ERK activation in a nuclear receptor–independent manner. For example, hydroxylated retinol metabolites directly bind PKCα with nanomolar affinity, and markedly enhance the activation of PKCα and the entire downstream ERK1/2 pathway in a redox-dependent manner [46]. We recently reported that tRA slightly suppressed both basal and oxidative stress–induced p38 phosphorylation in mesangial cells [20], although tRA has also been reported to induce p38 phosphorylation in leukemia cells [50]. tRA suppression of p38 phosphorylation in mesangial cells is likely due to the cell type–specific and RAR-dependent induction of MKP-1 [20]. tRA-induced p38 phosphorylation in NB-4 leukemia cells is also likely to be dependent on RAR since p38 phosphorylation was not induced in a RAR-deficient NB-4 variant [50]. In addition, tRA has both RAR-dependent and -independent suppressive effects on JNK activity in a number of cell types, including rat mesangial cells [20, 29]. Conversely, in P19 embryonal carcinoma cells, tRA induces PKC-dependent JNK activation, which is required for tRA-induced neural differentiation [47]. Finally, retinoids also affect some other kinases, such as protein kinase A and phosphatidylinositide 3-kinase/Akt [51]. The biological importance of retinoid-induced protein kinase activation and suppression has recently been reviewed by Bastien and Rochette-Egly, and notably, most of the aforementioned protein kinases modify the functions of retinoids in a reciprocal fashion by specifically phosphorylating retinoid nuclear receptors [51].

RETINOIDS IN RENAL DEVELOPMENT: PHYSIOLOGIC AND PHARMACOLOGIC IMPLICATIONS

Severe vitamin A deficiency–induced renal aplasia/hypoplasia, horseshoe kidney, and ureteral abnormalities were first reported half a century ago. More recently, it has become clear that even moderate vitamin A deficiency, which does not cause any significant abnormalities

in other organs, results in reduced nephron number [52]. Furthermore, combined knockout of any two RAR isotypes produces similar abnormalities to those observed in severe vitamin A deficiency [39]. It has been established that the malformation of both the kidney and the distal ureter observed in vitamin A deficiency and RAR double knockout models is due to loss of RA-induced expression of Ret in the developing ureteric bud, which mediates the epithelial/mesenchymal interactions and epithelial cell remodelling needed for nephron genesis and ureter maturation [53, 54]. However, the Ret gene is not a direct target for RA and its receptors [25]. Rather, its expression in the ureteric bud is controlled by an unidentified gene(s) in the stroma under the direct control of RA [53]. Therefore, it can be anticipated that investigation into the direct target genes affected by RA signalling will further advance our understanding of the role of retinoids in renal development. Midkine, sonic hedgehog, Hox d-11, matrix metalloproteinases, and tissue inhibitors of metalloproteinases have all been proposed as potential targets of interest [55], but their exact roles are still to be defined. For example, the midkine promoter contains a functional RARE [56], and midkine expression is reduced in vitamin A deficiency [57]. In in vitro studies, nephrogenesis was strongly inhibited in the presence of neutralizing antibodies for midkine; the number of nephrons formed in vitro was reduced by approximately 50% without changes in ureteric bud branching morphogenesis [57]. However, since midkine knockout mice have apparently normal kidney development [58], the role of midkine in renal development remains to be elucidated.

If malnutrition at a critical, sensitive period of early life has permanent effects on organ structure, physiology, and metabolism, and therefore, influences adult life and health, programming of the fetus may result from adaptations invoked when the materno-placental nutrient supply fails to match the fetal nutrient demand [59]. For example, fetal vitamin A deficiency also produces adult hypertension, probably due to water retention as a result of reduced nephron number in individuals who experienced fetal vitamin A deficiency [59].

The role of retinoids in renal development also has pharmacologic implications. Given the developmental role of retinoids in regulating stem cell differentiation and epithelial/mesenchymal interactions, it is rational to ask whether the effective mechanisms of retinoids in renal development are also operative in the amelioration of renal injury in acquired lesions [60]. Indeed, accumulating evidence shows that: the repair process in the kidney also involves stem cell differentiation, cell transdifferentiation, and epithelial/mesenchymal interactions [61]; retinoid deficiency retards renal repair in the PAN nephrosis model [6]; and human stem cells resemble human embryonal carcinoma cells [62], which have been known to be differentiated by retinoids. We have also noted that, in a recent report by Suzuki et al, the medium for differentiating bone marrow stem cells into mesangial-like cells contains tRA [63].

RETINOIDS IN THE PREVENTION AND TREATMENT OF RENAL AND URETERAL DISEASES

Importance of vitamin A in the defense against urinary tract infections

The susceptibility to urinary tract infections increases in vitamin A deficiency and decreases with supplementation of vitamin A [60]. Similarly, patients with a low serum vitamin A level have an increased susceptibility to chronic pyelonephritis-induced renal scarring, and the severity of renal scarring decreases with vitamin A supplementation in experimentally induced pyelonephritis in rats [60]. In the 5/6 nephrectomy model, characterized by focal segmental glomerulosclerosis, tubular degeneration, and interstitial fibrosis, low-dose vitamin A supplementation tends to reduce glomerular and interstitial injury, although high-dose vitamin A exacerbates injury in both compartments [60]. One explanation for the nephrotoxicity of vitamin A observed in 5/6 nephrectomized rats might be the excessive accumulation of vitamin A due to reduced kidney mass, since the kidney is one of the most important organs involved in vitamin A metabolism [64], and all the major limiting enzymes of vitamin A activation and detoxification (RALDH1-3) are expressed in the kidney [6].

Treatment of experimental mesangioproliferative nephritis and identification of mesangial cells as a target for RA

Wagner et al first investigated the effect of tRA and 13cRA on acute and chronic anti-Thy1.1 mesangioproliferative nephritis rat models [1, 3]. In the acute model, it was reported that RAs completely prevented hypertension and renal failure, dramatically reduced proteinuria, significantly reduced intraglomerular cell proliferation, capillary occlusion, macrophage infiltration, and fibrin deposition, while the expression of platelet-derived growth factor B-chain (PDGF-B), transforming growth factor (TGF) β1, and its receptor was inhibited [1, 65]. In the chronic model, 13cRA not only reduced blood pressure and albuminuria, but also prevented glomerulosclerosis, interstitial fibrosis, and macrophage infiltration [3]. The dramatic antihypertensive effect of RAs in nephritic rats might be due to the suppressive effect of RAs on the renin-angiotensin system (RAS). For example, tRA significantly reduced both serum angiotensin-converting enzyme (ACE) activity and mRNA levels of all the RAS components examined in the renal cortex [66]. Since tRA inhibits

angiotensin II–stimulated c-fos expression, downregulates the AT1 receptor, and antagonizes the functions of angiotensin II in vitro [66–68], it is likely that tRA also directly antagonizes angiotensin II. Indeed, 13cRA effectively prevented hypertension and intrarenal TGFβ expression induced by chronic angiotensin II infusion [abstract; Schade K et al, *J Am Soc Nephrol* 12:473A, 2001].

Wagner's group also used synthetic retinoid receptor–specific agonists to treat established chronic anti-Thy1.1 nephritic rats. They compared an RARα-selective agonist (AGN195183) and an RXR-selective agonist (AGN194204) and found that both had comparable effects on blood pressure, proteinuria, glomerulosclerosis index, glomerular macrophage infiltration, glomerular TGF-β1, and prepro-endothelin-1 (prepro-ET-1) expression and interstitial expansion [4]. In another series of experiments, a RAR agonist, arotinoid, a RXR agonist, Ro-257386, and an anti-AP-1 retinoid, BMS-453, were used to treat anti-Thy1.1 nephritis. These three compounds similarly suppressed glomerular cell proliferation and macrophage infiltration and inhibited glomerular ET-1 and ET-1 receptor expression, but differed in preventing hypertension, albuminuria, and renal failure. In summary, BMS-453 was most effective in reducing albuminuria and was moderately effective in preventing hypertension and renal failure; Ro-257386 most potently reduced blood pressure, moderately reduced proteinuria, but was not effective in restoring renal function; arotinoid was most effective in preventing renal failure, although it did not significantly prevent either hypertension or proteinuria [2].

Anti-Thy1.1 glomerulonephritis is induced by anti-Thy1.1 antibody, which specifically targets mesangial cells [1, 3]. One of the important contributions of the aforementioned animal studies is in providing in vivo evidence to support the idea that mesangial cells are a target for the therapeutic actions of retinoids [19], which has been suggested by the following in vitro findings: (*1*) both rat and human mesangial cells express all RAR and RXR isotypes [19, 20]; (*2*) RAs inhibit proliferation [19, 27], regulate apoptosis [19, 20, 28, 29, 33]; and suppress osteopontin, fibronectin [19], MCP-1 [34], and iNOS [38] expression in mesangial cells.

Treatment of puromycin aminonucleoside (PAN) nephrosis: Identification of podocytes as a target for RA and elucidation of the RA signal as a built-in feedback protection mechanism

PAN nephrosis, which resembles human minimal change nephrosis, is characterized by nephrotic syndrome and damage to podocytes [5]. Our recent work showed that tRA administered orally starting either on the day of PAN injection or two days after PAN injection almost completely prevented PAN-induced proteinuria. Delayed administration of tRA starting four days after PAN injection had no effect on the peak of proteinuria at day nine, but still reduced proteinuria to less than half of the untreated controls at day 16 [5]. These data indicate that tRA might have both prophylactic and therapeutic effects on PAN-induced kidney injury, possibly due to the ability of tRA to prevent PAN-induced apoptosis of podocytes and to suppress interstitial mononuclear cell infiltration and inhibit MCP-1 and fibronectin expression [5]. The efficacy of tRA in preventing PAN nephrosis is also supported by a recent report by Suzuki et al, showing that a vitamin A–deficient diet increased, while exogenous tRA suppressed, PAN-induced proteinuria, and tRA might promote the repair of the glomerular filtration barrier by specifically inducing the expression of nephrin in podocytes [6]. These investigators also reported that both RALDH2 and RARα were selectively and strongly expressed in podocytes, but not in endothelial and mesangial cells in PAN-nephrotic rats, indicating that podocytes are not only a major natural source of, but also a major target for, RAs in vivo [6]. This is further supported by our in vitro data that tRA prevents PAN-induced apoptosis in a podocyte cell line [5].

Treatment of experimental systemic lupus erythematosus (SLE) and influence of retinoids on immunity

We recently examined the effect of tRA on MRL/lpr mice, an animal model of human SLE, and found that tRA significantly reduced proteinuria, inhibited lymphoadenopathy and splenomegaly, prevented glomerular hypercellularity, mesangial expansion, and interstitial inflammatory infiltration, and inhibited RANTES (regulated on activation, normal T cells expressed and secreted), macrophage inflammatory protein-1 (MIP-1), and MCP-1 expression, although it affected neither plasma immunoglobulins (Igs), anti-DNA antibody, nor glomerular deposition of IgG and C3 [abstract; de Lema GP et al, *J Am Soc Nephrol* 13:F174A, 2002].

Kinoshita et al recently used NZB/NZW F1 mice, another murine SLE model, to examine the effects of tRA on experimental SLE [7]. Notably, in this model, at eight months of age, there was no significant change in renal histology, proteinuria, and mortality in the tRA-treated group, whereas most of the vehicle-treated animals developed significant glomerular, tubular, and interstitial changes, more than 50% developed significant proteinuria, and there was 40% mortality. tRA also significantly reduced the number of infiltrating T cells, B cells, and macrophages in the periglomerular, interstitial, and perivascular areas. tRA-treated mice had significantly less glomerular deposition of total IgG, IgG2a, and IgG2b, and significantly reduced serum levels of anti-DNA antibodies, especially the IgG2a

isotype. Splenomegaly was less marked, and the levels of interferon-γ (IFN-γ), interleukin-2 (IL-2), and IL-10 expression in splenic $CD4^+$ T cells, as well as the serum levels of these factors, were dramatically reduced in tRA-treated mice [7].

The seemingly conflicting findings in MRL/lpr mice and those in NZB/NZW F1 mice lie in the influence of tRA on immune complex deposition. Our studies showed that tRA suppressed renal injury without affecting glomerular deposition of IgG and C3 [abstract; de Lema GP et al, *J Am Soc Nephrol* 13:F174A, 2002]. In contrast, Kinoshita et al found that tRA significantly suppressed IgG deposition [7]. This can be explained by the isotype difference of the anti-DNA antibody IgG in these two models. In MRL/lpr mice, the major pathogenic circulating anti-DNA antibody, which is also deposited in the glomeruli, is of the IgG3 isotype [69]; however, in NZB/W F1 mice, the complement-fixing IgG2a anti-DNA antibody is predominantly nephritogenic [7]. In fact, tRA did not suppress plasma IgG3 and glomerular deposition of IgG3 even in NZB/NZW F1 mice, although the IgG2a anti-DNA antibody was significantly suppressed by tRA [7]. Therefore, it seems that tRA may protect the kidneys both upstream (as in NZB/NZW F1 mice) and downstream (as in MRL/lpr mice) of immune complex deposition.

Retinoids modulate the immune response by regulating a shift between the responses of Th1 and Th2 lymphocytes. In vivo findings that vitamin A deficiency changed the immune response from a Th2-type to a Th1-type response [70], and the in vitro observations that tRA and vitamin A inhibit Th1 cytokine production by T cells [71], are further supported by the findings of Kinoshita et al that tRA dramatically suppressed Th1 cytokines IFN-γ and IL-2, but not the Th2 cytokine IL-4 and immunoglobin isotypes IgG1 and IgG3 [7].

Intriguingly, retinoids are not only potent inhibitors of autoimmune injury, but also important mediators of normal immune activities against infection and play a role in the development of both T-helper cells and B cells [72]. Vitamin A treatment of SLE patients and healthy controls resulted in an enhancement of antibody-dependent cell-mediated cytotoxicity, natural killer activity, and blastogenic response to mitogens [73]. In acne patients, a synthetic retinoid, etretinate, also increased natural killer cell number and activity, whereas 13cRA significantly reduced natural killer cell number and activity [74]. One mechanism of the immunomodulation by retinoids is probably by influencing spontaneous and activation-induced apoptosis of T lymphocytes in a RAR- and RXR-dependent manner [75]. Interestingly, different RAR isotypes may have different roles in regulating the immune system, because selective activation of RARγ induces apoptosis of T cells, while specific stimulation of RARα prevents RARγ-dependent T-cell apoptosis [75]. Another recently identified mechanism of the immune-enhancing effects of retinoids is the RAR-dependent induction of T-cell proliferation and IL-2 secretion [76].

Amelioration of renal damage in experimental acute renal allograft rejection

It was reported some time ago that a synthetic retinoid, Ro23-6457, significantly prolonged the survival of rat cardiac allografts [77]. Recently, the effect of 13cRA on acute renal allograft rejection was examined in a rat model. 13cRA significantly reduced albuminuria and serum creatinine, and significantly prevented acute vascular injury, acute glomerular injury, glomerular monocyte, macrophage and cytotoxic T-cell infiltration, as well as suppressing tubulointerstitial cell proliferation and inflammatory cell infiltration [8]. These data further suggest a role for retinoids in transplantation immunosuppression.

Treatment of experimental anti-glomerular basement membrane (anti-GBM) glomerulonephritis

Rat anti-GBM glomerulonephritis is an analog of human type I crescentic glomerulonephritis. Datta et al reported that tRA, administered orally for two weeks at 5 mg/kg/day starting immediately before immunization with anti-GBM antibody, did not affect proteinuria in the anti-GBM glomerulonephritis model at day 14, although it markedly reduced intraglomerular cell proliferation, significantly suppressed the activity of the proinflammatory molecule, iNOS, and enhanced the expression of the anti-inflammatory molecule, TGF-β1, in glomeruli [15]. Recently, Oseto et al used a different strategy to treat anti-GBM glomerulonephritis rats. In rats given tRA, 30 mg/kg/day, starting two weeks after immunization with anti-GBM antibody, they found that: (*1*) tRA significantly reduced proteinuria and blood pressure; (*2*) tRA significantly reduced glomerular damage suppressing crescent formation and macrophage infiltration; and (*3*) tRA significantly reduced the expression of mRNAs for TNFα, IL-1β, PDGF, MCP-1, ICAM-1, TGFβ1, collagen I, as well as α-smooth muscle actin [14].

Both studies support the idea that tRA has anti-inflammatory effects in the anti-GBM nephritis model. The different effects of tRA on proteinuria in these two studies might be explained by the different tRA concentrations, the different experimental design, or differences in the timing of treatment. It is interesting that tRA increased TGF-β1 expression in the acute inflammatory phase of the disease model [15], but reduced TGF-β1 expression in the later stage of the disease [14]. Based on the dual functions of TGF-β on inflammation and fibrosis, it is possible that tRA conditionally influences TGF-β1 expression, namely increasing TGF-β1 in the acute inflammatory phase of nephritis to suppress inflammation,

and decreasing TGF-β1 in the later stage of the nephritis to prevent glomerulosclerosis and interstitial fibrosis. The conditional induction and suppression of TGF-β1 expression by tRA has also been reported in cultured kidney fibroblasts [78].

Treatment of glomerulosclerosis and interstitial fibrosis: Retinoids have direct antisclerotic and antifibrotic actions, and may directly target tubulointerstitial cells

In experimental models of glomerular diseases such as anti-Thy1.1 nephritis [3, 65] and PAN nephrosis [5], retinoids have been reported to reduce extracellular matrix (ECM) accumulation in glomeruli and the renal interstitium. However, since retinoids have strong anti-inflammatory effects on glomerular mesangial cells, podocytes and immune/inflammatory cells, two further questions need to be addressed: are the antifibrotic and antisclerotic effects independent of the anti-inflammatory effects, and are the antifibrotic effects on the interstitium independent of the beneficial effects on glomeruli?

Our studies on progressive glomerular sclerosis in aging rats, a non-nephritic model of renal fibrosis, suggest that tRA might well prevent glomerulosclerosis in a manner independent of inflammation [9, 10]. In these studies, 18-month-old male Fischer 344 rats were fed with standard chow with or without a low dose of tRA (1 mg/kg/day vs. 10 mg/kg/day for treatment of PAN nephrosis) for three months. tRA significantly increased the glomerular filtration rate [9] and suppressed TGF-β expression [10] in the renal cortex. Since glomerular hydrogen peroxide (H_2O_2) production increases in glomerulosclerosis and, in vitro, H_2O_2 induces apoptosis of mesangial cells [33], we hypothesize that tRA prevents loss of resident renal cells by inhibiting H_2O_2-induced apoptosis. Indeed, we found that tRA potently inhibits H_2O_2-induced apoptosis in both rat and human mesangial cells by induction of MKP-1 and inhibition of the JNK-AP-1 pathway [9, 10, 19, 20, 28, 29, 33].

tRA not only targets glomerular mesangial cells and podocytes, but also directly targets renal fibroblasts. As shown in Figure 2, our recent unpublished data indicated that tRA significantly prevented TGF-β1–induced fibronectin mRNA expression in human renal fibroblasts. These in vitro data, together with the demonstrated efficacy of tRA in treating unilateral ureter obstruction-induced tubulointerstitial nephritis [11], argue in favor of a direct protective effect of tRA on the tubulointerstitial injury, independent of any protective effects on the glomeruli, and lend support to our hypothesis that tRA has inflammation-independent antifibrotic actions. Other reported effects of retinoids on renal tubular and interstitial cells include: (*1*) tRA significantly stimulates renal tubular epithelial cell proliferation with [79] or without [80] significant hypertrophy, and therefore might be involved in the functional compensation, hypertrophy, and repair of renal tubules; (*2*) in normal rat kidney fibroblasts tRA promotes secretion of TGF-β2 and decreases TGF-β1 expression [78].

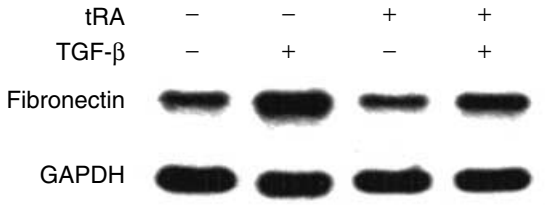

Fig. 2. Effects of tRA on TGFβ-induced fibronectin mRNA expression in human renal fibroblasts. Confluent human renal fibroblasts were made quiescent in DMEM:Ham's F12 (1:1) medium containing 0.1% fetal calf serum (FCS) for 48 hours before being treated with 5 ng/mL TGFβ1 for 24 hours and 48 hours in the absence or presence of 1 μmol/L tRA. Total RNA was extracted and subjected to Northern hybridization for fibronectin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs. A representative Northern blot of cells treated for 24 hours is shown (48-hour experiments show a similar trend; data not shown).

POTENTIAL ADVANTAGES OF RETINOIDS IN TREATMENT OF RENAL DISEASES

Based on the accumulating data, it is to be anticipated that retinoids might prove useful in the treatment of various renal conditions, some of which, such as hypertensive nephropathy, focal-segmental glomerulosclerosis, pyelonephritis, and renal interstitial fibrosis, are contraindicated in the use of glucocorticoids.

One of the most important adverse effects of glucocorticoids and cytotoxic agents is the risk of infection. In contrast, as shown in Table 1, retinoids have been used in both infective and noninfective renal inflammation, and have rarely been reported to induce severe acquired infection in patients. This might be due to the fact that the effect of retinoids on immunity is "immune modulation" rather than a nonspecific "immune suppression," as described in the above section.

High blood pressure and intrarenal activation of the renin-angiotensin and the endothelin systems are common findings in severe renal diseases. Retinoids can intervene in both these systems to reduce blood pressure. Furthermore, retinoids have protective effects on blood vessels and the heart in a blood pressure–independent manner [81, 82]. These effects contrast with those of glucocorticoids, which enhance blood pressure by inducing water retention and increasing plasma angiotensinogen concentration secondary to increased hepatic synthesis.

A procoagulative state is another important factor in glomerulonephritis. The conventional medication, glucocorticoids, induces hypercoagulation and hypofibrinolytic activity. In contrast, tRA has been shown to

induce the anticoagulation molecule, thrombomodulin, and the profibrinolytic enzyme, urokinase [83, 84], and to be effective in the treatment of endotoxin-induced disseminated intravascular coagulation (DIC) in rats [85].

Agonists of RAR and RXR, as well as selective anti-AP-1 retinoids, have been demonstrated to be effective in treating nephritic animals, although they might be differentially effective in preventing hypertension, proteinuria, and renal failure [2]. Since there are three isotypes of both RAR and RXR with overlapping and distinct functions, it is rational to ask whether it is possible to use retinoid nuclear receptor isotype-selective ligands for a given indication to minimize side effects. Indeed, tRA-induced skin irritation and cytotoxicity are largely due to the activation of RARγ, but not RARα, and therefore, a selective RARα agonist has been successfully used to reduce side effects [86, 87]. In keratinocytes, in which there is no expression of RARβ, gene knockout of RARα and γ induces distinctive changes in the pattern of gene expression, further suggesting that RAR α and γ have unique functions [88]. In mesangial cells, which express all RAR isotypes, RARβ has a distinctive role in regulating MKP-1 expression when compared with RAR α and γ [20]. These data suggest that isotype-selective RAR agonists may have different therapeutic efficacy with varying side effects.

Another advantage of retinoids in the treatment of renal diseases in man compared with other new medications is that retinoids have been used to treat other diseases for many years; hence, information on the side effects of retinoids such as tRA and 13cRA is readily available. In the treatment of leukemia, combined use of retinoids and glucocorticoids has been shown to have synergistic effects in inducing differentiation and inhibiting proliferation of leukemia cells and to reduce major adverse effects [89]. It is possible that, in view of their distinctive mechanisms of actions and given the synergistic inhibition of mesangial cell proliferation in vitro [abstract; Kaname S et al, *J Am Soc Nephrol* 13:297A, 2002], retinoids and glucocorticoids also synergize in the treatment of renal diseases.

POTENTIAL LIMITATIONS OF RETINOIDS IN TREATMENT OF RENAL DISEASES

Reported side effects of retinoids

Although most studies in rodents have found few side effects of retinoids, a number of major side effects in patients besides some minor ones, such as elevated serum lipids, generalized xerosis, and alopecia, have been documented in the literature. For instance, the well-known teratogenic effect of retinoids may lead to severe birth defects, and it has been proposed that contraception should be used during retinoid therapy and for two years afterwards [90]. Another important side effect is the risk of retinoids to the central nervous system. For example, in animal studies, 13cRA has been shown to suppress hippocampal cell division and hippocampal-dependent learning [91], and reports of intracranial hypertension, depression, and suicidal ideation with retinoid use have prompted an examination of their serious and life-threatening potential [90]. Finally, induction therapy of acute promyelocytic leukemia with tRA is standard, despite significant side effects, of which the most important is "RA syndrome," in which there is a hyperinflammatory reaction with capillary leakage, infiltration of myeloid cells into internal organs, and systemic signs of inflammation. Where "RA syndrome" occurs, administration of tRA must be terminated and replaced by corticosteroids. Fortunately, since "RA syndrome" is a syndrome of tissue infiltration by maturing myeloid cells, which only occurs during induction treatment of leukemia with tRA or other medications, the syndrome is specific to leukemia patients, even in the absence of tRA treatment [92], and it is unlikely that it will be a significant side effect of tRA use in nephritis patients.

Evidence of the proinflammatory effects of retinoids

Although the severe inflammatory "RA syndrome" is unlikely to happen in renal patients treated with retinoids, the possibility that retinoids might have proinflammatory effects in patients with renal diseases cannot be excluded. Emerging evidence indicates that, although retinoids are potent anti-inflammatory agents, they may also have proinflammatory potential by inducing some proinflammatory molecules. For example, retinoids can induce iNOS expression in vivo [93], and enhance IL-8 [94] and ICAM-1 [37] expression in vitro. Further, since tRA has been shown to potentiate tubulointerstitial nephritis both in patients and in animal models [16, 17], retinoids may also have proinflammatory effects on renal tubulointerstitial cells. In support of this hypothesis, midkine, a RA-inducible gene product, is reported to be involved in neutrophil infiltration in the tubulointerstitium in ischemic renal injury [95].

We also found that retinoids only have suppressive effects on selected inflammatory mediators induced by selected stimuli. For example, although tRA suppresses the constitutive expression of MCP-1 in rat mesangial cells, it does not affect IL-1β–induced MCP-1 expression [34]. Further, tRA and 9cRA did not suppress, but rather enhanced, the expression of MIF, COX-1, COX-2, and VCAM-1 in human and rat mesangial cells [abstract; Xu Q et al, *J Am Soc Nephrol* 14:86A, 2003]. The biological significance of RA-induced expression of these proinflammatory molecules is not clear; we speculate that the RA-inducible pro-inflammatory molecules might be related to the resistance to and proinflammatory side effects of tRA (Fig. 3). Interestingly, although tRA has some advantages compared to glucocorticoids

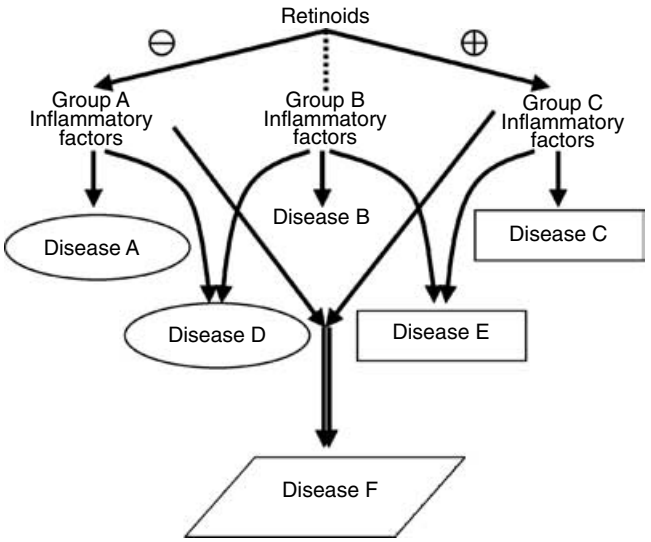

Fig. 3. Different effects of retinoids on varying groups of proinflammatory factors might contribute to the different responses of renal diseases to retinoid treatment. Inflammatory factors are theoretically divided into three groups, which respond differentially to RA treatment, namely, RAs suppress group A, induce group C, and have little influence on group B. Consequently, the group A and group D diseases that are mediated by group A or group A plus group B inflammatory factors are good indicators for RA treatment; the group B diseases that are solely mediated by group B inflammatory factors are not responsive to RAs; the group C and group E diseases that are mediated by group C inflammatory factors only or by group C plus group B factors are not indicators for retinoid treatment, and may even be exacerbated by retinoids. The group F diseases that are mediated by both group A and group C inflammatory factors may become resistant to RAs, or may respond to retinoids, but at the same time develop some inflammatory side effects. The solid line with a circled 'minus' indicates a suppression; the solid line with a circled 'plus' stands for induction. The dashed line indicates no interaction.

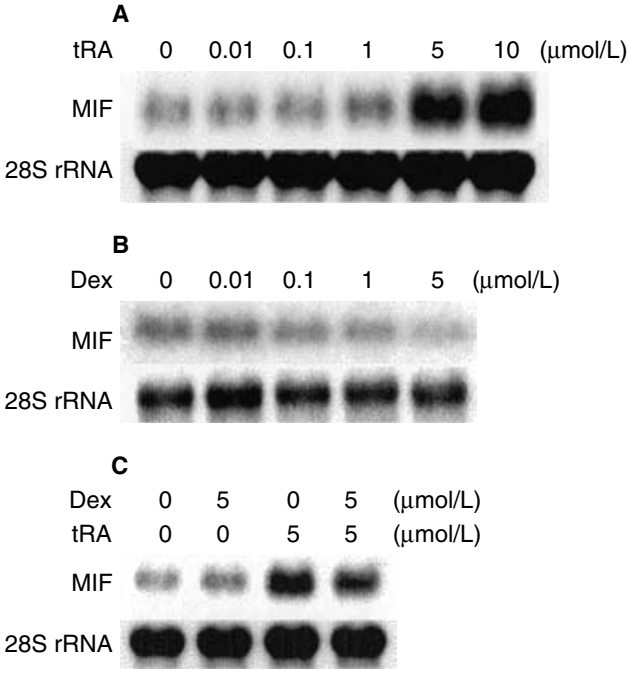

Fig. 4. The effects of tRA and dexamethasone (Dex) on MIF mRNA expression in human mesangial cells. Cultured human glomerular mesangial cells were made quiescent in RPMI 1640 medium containing 0.5% FCS for 24 hours prior to treatment with (*A*) 0 to 10 μmol/L tRA, (*B*) 0 to 5 μmol/L Dex, or (*C*) 0 to 5 μmol/L tRA and/or Dex for 24 hours. Total RNA was extracted and subject to Northern hybridization for MIF mRNA and 28S ribosomal RNA (rRNA). A representative Northern blot analysis is shown.

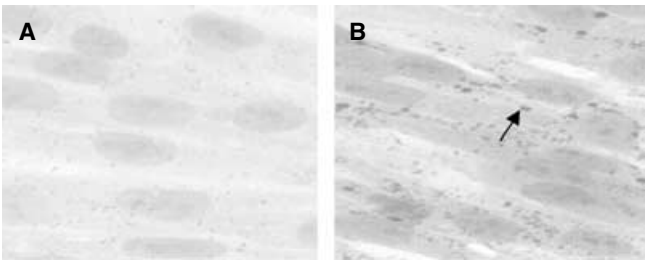

Fig. 5. tRA increased LDL accumulation in pig smooth muscle cells: Oil Red O staining. Pig artery smooth muscle cells were cultured in RPMI 1640 medium containing 1% FCS for 24 hours and followed by a further 24 hours in RPMI 1640, 1% FCS supplemented with 200 μg/mL LDL in the absence (*A*) or presence (*B*) of 5 μmol/L tRA. Cells were washed three times with cold phosphate-buffered saline, fixed with 5% formalin and subjected to Oil Red O staining. The arrow in (B) points to an LDL droplet visualized by Oil Red O staining.

(as described in the previous section), our recent unpublished data showed that tRA-induced MIF expression in cultured human mesangial cells can be inhibited by dexamethasone (Fig. 4), suggesting that, just as in the case of "RA syndrome," glucocorticoids might be useful to antagonize the proinflammatory side effects of tRA.

Do retinoids have atherogenic potential?

Despite the recent reports that tRA reduced the early neointimal proliferation and artery restenosis in a balloon angioplasty model of rabbit [96], and that RXR agonists prevented hyperlipidemia-induced atherosclerosis [97], we have found that tRA dramatically induces low-density lipoprotein (LDL) accumulation in smooth muscle cells [abstract; Xu Q et al, *J Am Soc Nephrol* 14:86A, 2003] (Fig. 5). In addition, tRA has also been reported to induce oxidized LDL accumulation in macrophages by inducing CD36 expression in a RAR-dependent manner [98]. Since LDL accumulation in smooth muscle cells and macrophages is central to the pathogenesis of atherosclerosis, and given that human atherosclerotic plaques contain 10-fold increased concentrations of RA and have activated RAR expression [98], it is important to determine whether long-term use of retinoids will increase the mortality from cardio- and cerebrovascular diseases.

CONCLUSION AND PERSPECTIVES

Retinoids play an important role in renal development, and nephrogenic hypertension might be programmed by

fetal vitamin A deficiency, a common condition in pregnant women [59, 99]. Therefore, maintaining an adequate vitamin A supply and liver reserve in pregnant women should prevent vitamin A deficiency in the fetus, and might help prevent hypertension and renal diseases in adults. Paradoxically, too much vitamin A can also severely affect normal development, although excessive dietary intake of vitamin A has been associated with teratogenicity in fewer than 20 individuals in over 30 years [99]. Therefore, vitamin A supplementation should be approached with caution, taking into account the estimated vitamin A status of a pregnant woman and whether supplementation can be appropriately supervised. On the other hand, it might also be very harmful for pregnant women to simply abstain from foods rich in vitamin A in order to avoid the potential teratogenicity of vitamin A. It is advisable to routinely monitor the liver reserve, serum, and milk vitamin A levels in pregnant women so that both hypo- and hypervitaminosis A in fetus can be prevented [100].

In the adult, retinoids target mesangial cells, podocytes, tubular epithelial cells, interstitial fibroblasts, as well as T lymphocytes and macrophages, and have anti-inflammatory, anticoagulatory, antifibrotic effects and proliferation- and immunity-modulating actions. All these features make retinoids a promising new generation of renal medication for use in a variety of renal diseases.

The pharmacologic effects of retinoids reported in animal studies suggest that it may be worthwhile to examine whether retinoids can be useful in dealing with the following conditions: (*1*) steroid-resistant glomerulonephritides, such as focal segmental glomerulosclerosis, membranous nephropathy, and membranous proliferative glomerulonephritis; (*2*) glomerulonephritis responsive to but developing secondary resistance to steroids, such as refractory minimal change nephrosis, mesangioproliferative glomerulonephritis; (*3*) glomerulonephritis with mild to moderate chronic renal failure and renal fibrosis; (*4*) glomerulonephritis complicated by diabetes or other side effects of steroids; (*5*) crescentic glomerulonephritis; (*6*) SLE and lupus nephritis; (*7*) renal transplantation. We suggest that comprehensive clinical trials of retinoid treatment of renal diseases should be carried out to further examine the therapeutic potential of retinoids.

Given the reduced incidence of "RA syndrome" and the suppression of tRA-induced proinflammatory molecule expression with the combined use of retinoids and steroids, it will also be important to establish whether combination therapy using lower doses of both compounds can achieve better therapeutic outcomes than either compound alone. In vitro studies to directly compare the effects of steroids, retinoids, or combination of the two on kidney cells and immune cells exposed to different stimuli will provide further insights into the advantages and disadvantages of these two groups of compounds and their combined use.

Any future clinical trials will have to provide careful monitoring of both major side effects, such as teratogenicity and depression, and other minor side effects. Although retinoids are unlikely to induce "RA syndrome" in patients with renal disease, retinoid induction of proinflammatory molecules in mesangial cells suggests that primary and secondary resistance and inflammatory side effects may occur. In view of the potential of tRA to induce LDL and modified LDL accumulation in smooth muscle cells and macrophages, careful examination will be required to determine whether, among other adverse effects, retinoids have atherogenic potential. Finally, it remains to be tested whether RAR and RXR isotype-selective retinoids increase the benefit/risk ratio in kidney diseases.

ACKNOWLEDGMENTS

This review and our related work are supported by grants from the British Heart Foundation to Q.X. and from the Wellcome Trust to L.G.F. and M.K. It is also partly supported by the Creative Research Group Fund from NSFC (30121005) and the 973 program (G2000057003) to Q.X. The authors regret that because of space limitations, many important contributions could not be cited.

Reprint requests to Qihe Xu, M.D., Ph.D., Centre for Nephrology, Department of Medicine, in the Department of Biochemistry and Molecular Biology (2nd floor), Royal Free and University College Medical School, Royal Free Campus, Rowland Hill Street, London NW3 2PF, United Kingdom.
E-mail: q.xu@ucl.ac.uk

REFERENCES

1. Wagner J, Dechow C, Morath C, *et al*: Retinoic acid reduces glomerular injury in a rat model of glomerular damage. *J Am Soc Nephrol* 11:1479–1487, 2000
2. Lehrke I, Schaier M, Schade K, *et al*: Retinoid receptor-specific agonists alleviate experimental glomerulonephritis. *Am J Physiol Renal Physiol* 282:F741–F751, 2002
3. Schaier M, Lehrke I, Schade K, *et al*: Isotretinoin alleviates renal damage in rat chronic glomerulonephritis. *Kidney Int* 60:2222–2234, 2001
4. Schaier M, Liebler S, Schade K, *et al*: Retinoic acid α and retinoid × receptor specific agonists reduce renal injury in established chronic glomerulonephritis of the rat. *J Mol Med* 82:116–125, 2004
5. Moreno-Manzano V, Mampaso F, Sepulveda-Munoz JC, *et al*: Retinoids as a potential treatment for experimental puromycin-induced nephrosis. *Br J Pharmacol* 139:823–831, 2003
6. Suzuki A, Ito T, Imai E, *et al*: Retinoids regulate the repairing process of the podocytes in puromycin aminonucleoside-induced nephrotic rats. *J Am Soc Nephrol* 14:981–991, 2003
7. Kinoshita K, Yoo BS, Nozaki Y, *et al*: Retinoic acid reduces autoimmune renal injury and increases survival in NZB/W F(1) mice. *J Immunol* 170:5793–5798, 2003
8. Kiss E, Adams J, Grone HJ, *et al*: Isotretinoin ameliorates renal damage in experimental acute renal allograft rejection. *Transplantation* 76:480–489, 2003
9. Moreno-Manzano V, Rodriguez-Puyol M, Arribas-Gomez I, *et al*: Prevention by tretinoin (a*ll-trans*-retinoic acid) of age-related renal changes. *Int J Vitam Nutr Res* 67:427–431, 1997
10. Manzano VM, Puyol MR, Puyol DR, Lucio-Cazana J: Tretinoin prevents age-related renal changes and stimulates antioxidant defenses in cultured renal mesangial cells. *J Pharmacol Exp Ther* 289:123–132, 1999

11. Schaier M, Jocks T, Grone HJ, *et al*: Retinoid agonist isotretinoin ameliorates obstruction renal injury. *J Urol* 170:1398–1402, 2003
12. Kavukcu S, Soylu A, Turkmen M, *et al*: The role of vitamin A in preventing renal scarring secondary to pyelonephritis. *BJU Int* 83:1055–1059, 1999
13. Bennett RT, Mazzaccaro RJ, Chopra N, *et al*: Suppression of renal inflammation with vitamins A and E in ascending pyelonephritis in rats. *J Urol* 161:1681–1684, 1999
14. Oseto S, Moriyama T, Kawada N, *et al*: Therapeutic effect of all-*trans* retinoic acid on rats with anti-GBM antibody glomerulonephritis. *Kidney Int* 64:1241–1252, 2003
15. Datta PK, Reddy RS, Lianos EA: Effects of all-*trans*-retinoic acid (atRA) on inducible nitric oxide synthase (iNOS) activity and transforming growth factor β-1 production in experimental anti-GBM antibody-mediated glomerulonephritis. *Inflammation* 25:351–359, 2001
16. Tomita N, Kanamori H, Fujita H, *et al*: Granulomatous tubulointerstitial nephritis induced by all-*trans* retinoic acid. *Anticancer Drugs* 12:677–680, 2001
17. Moulder JE, Fish BL, Regner KR, *et al*: Retinoic acid exacerbates experimental radiation nephropathy. *Radiat Res* 157:199–203, 2002
18. Turman MA, Hammond S, Grovas A, Rauck AM: Possible association of retinoic acid with bone marrow transplant nephropathy. *Pediatr Nephrol* 13:755–158, 1999
19. Manzano VM, Munoz JC, Jimenez JR, *et al*: Human renal mesangial cells are a target for the anti-inflammatory action of 9-*cis* retinoic acid. *Br J Pharmacol* 131:1673–1683, 2000
20. Xu Q, Konta T, Furusu A, *et al*: Transcriptional induction of mitogen-activated protein kinase phosphatase 1 by retinoids. Selective roles of nuclear receptors and contribution to the anti-apoptotic effect. *J Biol Chem* 277:41693–41700, 2002
21. Ross SA, McCaffery PJ, Drager UC, De Luca LM: Retinoids in embryonal development. *Physiol Rev* 80:1021–1054, 2000
22. Marill J, Idres N, Capron CC, *et al*: Retinoic acid metabolism and mechanism of action: A review. *Curr Drug Metab* 4:1–10, 2003
23. Niederreither K, Abu-Abed S, Schuhbaur B, *et al*: Genetic evidence that oxidative derivatives of retinoic acid are not involved in retinoid signaling during mouse development. *Nat Genet* 31:84–88, 2002
24. Gronemeyer H, Miturski R: Molecular mechanisms of retinoid action. *Cell Mol Biol Lett* 6:3–52, 2001
25. Balmer JE, Blomhoff R: Gene expression regulation by retinoic acid. *J Lipid Res* 43:1773–1808, 2002
26. Germain P, Iyer J, Zechel C, Gronemeyer H: Co-regulator recruitment and the mechanism of retinoic acid receptor synergy. *Nature* 415:187–192, 2002
27. Simonson MS: Anti-AP-1 activity of all-*trans* retinoic acid in glomerular mesangial cells. *Am J Physiol* 267:F805–F815, 1994
28. Moreno-Manzano V, Ishikawa Y, Lucio-Cazana J, Kitamura M: Suppression of apoptosis by all-*trans*-retinoic acid. Dual intervention in the c-Jun N-terminal kinase-AP-1 pathway. *J Biol Chem* 274:20251–20258, 1999
29. Konta T, Xu Q, Furusu A, *et al*: Selective roles of retinoic acid receptor and retinoid × receptor in the suppression of apoptosis by all-*trans*-retinoic acid. *J Biol Chem* 276:12697–12701, 2001
30. Suzukawa K, Colburn NH: AP-1 trans-repressing retinoic acid does not deplete coactivators or AP-1 monomers but may target specific Jun or Fos containing dimers. *Oncogene* 21:2181–2190, 2002
31. Benkoussa M, Brand C, Delmotte MH, *et al*: Retinoic acid receptors inhibit AP1 activation by regulating extracellular signal-regulated kinase and CBP recruitment to an AP1-responsive promoter. *Mol Cell Biol* 22:4522–1534, 2002
32. Lin F, Xiao D, Kolluri SK, Zhang X: Unique anti-activator protein-1 activity of retinoic acid receptor β. *Cancer Res* 60:3271–3280, 2000
33. Xu Q, Konta T, Kitamura M: Retinoic acid regulation of mesangial cell apoptosis. *Exp Nephrol* 10:171–175, 2002
34. Lucio-Cazana J, Nakayama K, Xu Q, *et al*: Suppression of constitutive but not IL-1β-inducible expression of monocyte chemoattractant protein-1 in mesangial cells by retinoic acids: Intervention in the activator protein-1 pathway. *J Am Soc Nephrol* 12:688–694, 2001
35. Na SY, Kang BY, Chung SW, *et al*: Retinoids inhibit interleukin-12 production in macrophages through physical associations of retinoid × receptor and NFkappaB. *J Biol Chem* 274:7674–7680, 1999
36. Na SY, Kim HJ, Lee SK, *et al*: IkappaBβ interacts with the retinoid × receptor and inhibits retinoid-dependent transactivation in lipopolysaccharide-treated cells. *J Biol Chem* 273:3212–3215, 1998
37. Chadwick CC, Shaw LJ, Winneker RC: TNF-α and 9-*cis*-retinoic acid synergistically induce ICAM-1 expression: Evidence for interaction of retinoid receptors with NF-kappa B. *Exp Cell Res* 239:423–429, 1998
38. Datta PK, Lianos EA: Retinoic acids inhibit inducible nitric oxide synthase expression in mesangial cells. *Kidney Int* 56:486–493, 1999
39. Kastner P, Mark M, Chambon P, *et al*: Nonsteroid nuclear receptors: What are genetic studies telling us about their role in real life? *Cell* 83:859–869, 1995
40. Kang JX, Bell J, Beard RL, Chandraratna RA: Mannose 6-phosphate/insulin-like growth factor II receptor mediates the growth-inhibitory effects of retinoids. *Cell Growth Differ* 10:591–600, 1999
41. Shaw N, Elholm M, Noy N: Retinoic acid is a high affinity selective ligand for PPARβ/δ. *J Biol Chem* 278:41589–41592, 2003
42. Stehlin-Gaon C, Willmann D, Zeyer D, *et al*: All-*trans* retinoic acid is a ligand for the orphan nuclear receptor RORβ. *Nat Struct Biol* 10:820–825, 2003
43. Bayon Y, Ortiz MA, Lopez-Hernandez FJ, *et al*: Inhibition of IkappaB kinase by a new class of retinoid-related anticancer agents that induce apoptosis. *Mol Cell Biol* 23:1061–1074, 2003
44. Radominska-Pandya A, Chen G, Czernik PJ, *et al*: Direct interaction of all-*trans*-retinoic acid with protein kinase C (PKC). Implications for PKC signaling and cancer therapy. *J Biol Chem* 275:22324–22330, 2000
45. Cho Y, Klein MG, Talmage DA: Distinct functions of protein kinase Cα and protein kinase Cβ during retinoic acid-induced differentiation of F9 cells. *Cell Growth Differ* 9:147–154, 1998
46. Imam A, Hoyos B, Swenson C, *et al*: Retinoids as ligands and coactivators of protein kinase Cα. *FASEB J* 15:28–30, 2001
47. Wang H, Ikeda S, Kanno S, *et al*: Activation of c-Jun amino-terminal kinase is required for retinoic acid-induced neural differentiation of P19 embryonal carcinoma cells. *FEBS Lett* 503:91–96, 2001
48. Kambhampati S, Li Y, Verma A, *et al*: Activation of protein kinase C-δ (PKC-δ) by all-*trans*-retinoic acid. *J Biol Chem* 278:32544–32551, 2003
49. Hong HY, Varvayanis S, Yen A: Retinoic acid causes MEK-dependent RAF phosphorylation through RARα plus RXR activation in HL-60 cells. *Differentiation* 68:55–66, 2001
50. Alsayed Y, Uddin S, Mahmud N, *et al*: Activation of Rac1 and the p38 mitogen-activated protein kinase pathway in response to all-*trans*-retinoic acid. *J Biol Chem* 276:4012–4019, 2001
51. Bastien J, Rochette-Egly C: Nuclear retinoid receptors and the transcription of retinoid-target genes. *Gene* 328:1–16, 2004
52. Lelievre-Pegorier M, Vilar J, Ferrier ML, *et al*: Mild vitamin A deficiency leads to inborn nephron deficit in the rat. *Kidney Int* 54:1455–1462, 1998
53. Batourina E, Choi C, Paragas N, *et al*: Distal ureter morphogenesis depends on epithelial cell remodeling mediated by vitamin A and Ret. *Nat Genet* 32:109–115, 2002
54. Batourina E, Gim S, Bello N, *et al*: Vitamin A controls epithelial/mesenchymal interactions through Ret expression. *Nat Genet* 27:74–78, 2001
55. Burrow CR: Retinoids and renal development. *Exp Nephrol* 8:219–225, 2000
56. Matsubara S, Take M, Pedraza C, Muramatsu T: Mapping and characterization of a retinoic acid-responsive enhancer of midkine, a novel heparin-binding growth/differentiation factor with neurotrophic activity. *J Biochem* (Tokyo) 115:1088–1096, 1994
57. Vilar J, Lalou C, Duong VH, *et al*: Midkine is involved in kidney development and in its regulation by retinoids. *J Am Soc Nephrol* 13:668–676, 2002
58. Nakamura E, Kadomatsu K, Yuasa S, *et al*: Disruption of the midkine gene (Mdk) resulted in altered expression of a calcium

binding protein in the hippocampus of infant mice and their abnormal behaviour. *Genes Cells* 3:811–822, 1998
59. Moritz KM, Dodic M, Wintour EM: Kidney development and the fetal programming of adult disease. *Bioessays* 25:212–220, 2003
60. Kavukcu S, Turkmen MA, Soylu A: Could the effective mechanisms of retinoids on nephrogenesis be also operative on the amelioration of injury in acquired renal lesions? *Pediatr Nephrol* 16:689–690, 2001
61. Mene P, Polci R, Festuccia F: Mechanisms of repair after kidney injury. *J Nephrol* 16:186–195, 2003
62. Draper JS, Pigott C, Thomson JA, Andrews PW: Surface antigens of human embryonic stem cells: Changes upon differentiation in culture. *J Anat* 200:249–258, 2002
63. Suzuki A, Iwatani H, Ito T, *et al*: Platelet-derived growth factor plays a critical role to convert bone marrow cells into glomerular mesangial-like cells. *Kidney Int* 65:15–24, 2004
64. Kakela A, Kakela R, Hyvarinen H: Importance of the kidneys in metabolism of vitamins A1 and A2 and their fatty acyl esters in mink feeding on fish-based diets and exposed to Aroclor 1242. *Toxicol Appl Pharmacol* 187:118–127, 2003
65. Morath C, Dechow C, Lehrke I, *et al*: Effects of retinoids on the TGF-β system and extracellular matrix in experimental glomerulonephritis. *J Am Soc Nephrol* 12:2300–2309, 2001
66. Dechow C, Morath C, Peters J, *et al*: Effects of all-*trans* retinoic acid on renin-angiotensin system in rats with experimental nephritis. *Am J Physiol Renal Physiol* 281:F909–F919, 2001
67. Takeda K, Ichiki T, Funakoshi Y, *et al*: Downregulation of angiotensin II type 1 receptor by all-*trans* retinoic acid in vascular smooth muscle cells. *Hypertension* 35:297–302, 2000
68. Haxsen V, Adam-Stitah S, Ritz E, Wagner J: Retinoids inhibit the actions of angiotensin II on vascular smooth muscle cells. *Circ Res* 88:637–644, 2001
69. Takahashi S, Nose M, Sasaki J, *et al*: IgG3 production in MRL/lpr mice is responsible for development of lupus nephritis. *J Immunol* 147:515–519, 1991
70. Cantorna MT, Nashold FE, Hayes CE: In vitamin A deficiency multiple mechanisms establish a regulatory T helper cell imbalance with excess Th1 and insufficient Th2 function. *J Immunol* 152:1515–1522, 1994
71. Cantorna MT, Nashold FE, Chun TY, Hayes CE: Vitamin A down-regulation of IFN-γ synthesis in cloned mouse Th1 lymphocytes depends on the CD28 costimulatory pathway. *J Immunol* 156:2674–2679, 1996
72. Stephensen CB: Vitamin A, infection, and immune function. *Annu Rev Nutr* 21:167–192, 2001
73. Gergely P, Csaky L, Gonzalez-Cabello P: Immunological effects of retinoids. *Tokai J Exp Clin Med* 15:235–239, 1990
74. McKerrow KJ, MacKie RM, Lesko MJ, Pearson C: The effect of oral retinoid therapy on the normal human immune system. *Br J Dermatol* 119:313–320, 1988
75. Szondy Z, Reichert U, Fesus L: Retinoic acids regulate apoptosis of T lymphocytes through an interplay between RAR and RXR receptors. *Cell Death Differ* 5:4–10, 1998
76. Ertesvag A, Engedal N, Naderi S, Blomhoff HK: Retinoic acid stimulates the cell cycle machinery in normal T cells: Involvement of retinoic acid receptor-mediated IL-2 secretion. *J Immunol* 169:5555–5563, 2002
77. Orosz CG, Zinn NE, Bishop DK, *et al*: Analysis of retinoid-mediated immunosuppression in vivo. Effects of Ro23-6457 on cellular alloimmune responses. *Immunopharmacology* 22:49–58, 1991
78. Danielpour D, Kim KY, Winokur TS, Sporn MB: Differential regulation of the expression of transforming growth factor-βs 1 and 2 by retinoic acid, epidermal growth factor, and dexamethasone in NRK-49F and A549 cells. *J Cell Physiol* 148:235–244, 1991
79. Argiles A, Kraft NE, Hutchinson P, *et al*: Retinoic acid affects the cell cycle and increases total protein content in epithelial cells. *Kidney Int* 36:954–959, 1998
80. Anderson RJ, Ray CJ, Hattler BG: Retinoic acid regulation of renal tubular epithelial and vascular smooth muscle cell function. *J Am Soc Nephrol* 9:773–781, 1998
81. Shindo T, Manabe I, Fukushima Y, *et al*: Kruppel-like zinc-finger transcription factor is a target for angiotensin II signaling and an essential regulator of cardiovascular remodeling. *Nat Med* 8:856–863, 2002
82. Lu L, Yao T, Zhu YZ, *et al*: Chronic all-*trans* retinoic acid treatment prevents medial thickening of intramyocardial- and intrarenal arteries in spontanously hypertensive rats. *Am J Physiol Heart Circ Physiol* 285:H1370–H1377, 2003
83. Horie S, Ishii H, Matsumoto F, *et al*: Acceleration of thrombomodulin gene transcription by retinoic acid: Retinoic acid receptors and Sp1 regulate the promoter activity through interactions with two different sequences in the 5′-flanking region of human gene. *J Biol Chem* 276:2440–2450, 2001
84. Shimada J, Suzuki Y, Kim SJ, *et al*: Transactivation via RAR/RXR-Sp1 interaction: Characterization of binding between Sp1 and GC box motif. *Mol Endocrinol* 15:1677–1692, 2001
85. Aoshima K, Asakura H, Yamazaki M, *et al*: Treatment of disseminated intravascular coagulation (DIC) with all-*trans* retinoic acid in an endotoxin-induced rat model. *Semin Thromb Hemost* 24:227–231, 1998
86. Standeven AM, Teng M, Chandraratna RA: Lack of involvement of retinoic acid receptor α in retinoid-induced skin irritation in hairless mice. *Toxicol Lett* 92:231–240, 1997
87. Chen S, Ostrowski J, Whiting G, *et al*: Retinoic acid receptor γ mediates topical retinoid efficacy and irritation in animal models. *J Invest Dermatol* 104:779–783, 1995
88. Goyette P, Feng Chen C, *et al*: Characterization of retinoic acid receptor-deficient keratinocytes. *J Biol Chem* 275:16497–16505, 2000
89. Hicsonmez G: Retinoic acid and steroid in acute promyelocytic leukemia. *Leukemia* 17:1203, 2003
90. O'Donnell J: Overview of existing research and information linking isotretinoin (accutane), depression, psychosis, and suicide. *Am J Ther* 10:148–159, 2003
91. Crandall J, Sakai Y, Zhang J, *et al*: 13-cis-retinoic acid suppresses hippocampal cell division and hippocampal-dependent learning in mice. *Proc Natl Acad Sci USA* 101:5111–5116, 2004
92. De Botton S, Dombret H, Sanz M, *et al*: Incidence, clinical features, and outcome of all trans-retinoic acid syndrome in 413 cases of newly diagnosed acute promyelocytic leukemia. The European APL Group. *Blood* 92:2712–2718, 1998
93. Seguin-Devaux C, Devaux Y, Latger-Cannard V, *et al*: Enhancement of the inducible NO synthase activation by retinoic acid is mimicked by RARα agonist in vivo. *Am J Physiol Endocrinol Metab* 283:E525–E535, 2002
94. Chang MMJ, Harper R, Hyde DM, Wu R: A novel mechanism of retinoic acid-enhanced interleukin-8 gene expression in airway epithelium. *Am J Respir Cell Mol Biol* 22:502–510, 2000
95. Sato W, Kadomatsu K, Yuzawa Y, *et al*: Midkine is involved in neutrophil infiltration into the tubulointerstitium in ischemic renal injury. *J Immunol* 167:3463–3469, 2001
96. Herdeg C, Oberhoff M, Baumbach A, *et al*: Effects of local all-*trans*-retinoic acid delivery on experimental atherosclerosis in the rabbit carotid artery. *Cardiovasc Res* 57:544–553, 2003
97. Claudel T, Leibowitz MD, Fievet C, *et al*: Reduction of atherosclerosis in apolipoprotein E knockout mice by activation of the retinoid × receptor. *Proc Natl Acad Sci USA* 98:2610–2615, 2001
98. Wuttge DM, Romert A, Eriksson U, *et al*: Induction of CD36 by all-*trans* retinoic acid: Retinoic acid receptor signaling in the pathogenesis of atherosclerosis. *FASEB J* 15:1221–1223, 2001
99. Azais-Braesco V, Pascal G: Vitamin A in pregnancy: Requirements and safety limits. *Am J Clin Nutr* 71:1325S–1333S, 2000
100. Tanumihardjo SA: Assessing vitamin A status: Past, present and future. *J Nutr* 134:290S–293S, 2004