Evidence has been provided that the immunological mechanism is involved in the genesis or maintenance of hypertension. In the present study, we investigated the effects of interferon gamma, a potent immunomodulator derived from lymphocytes, on hypertension and organ damage in Dahl salt-sensitive rats and in spontaneously hypertensive rats. Subcutaneous injection of interferon gamma (5×10^4 units/kg body wt once a week for 10 weeks) reduced blood pressure in Dahl salt-sensitive rats fed a 4% high salt diet (174 versus 194 mm Hg, p<0.025). This blood pressure reduction was associated with an improvement of renal functions, an increase in glomerular filtration rate (690 versus 569 ml/day/100 g body wt, p<0.05), and decreases in urinary protein excretion (48 versus 78 mg/day/100 g body wt, p<0.0025) and urinary N-acetyl-β-D-glucosaminidase excretion (143 versus 183 milliunits/day/100 g body wt, p<0.05). Morphological investigation showed a marked resolution of the vascular injuries seen in untreated Dahl salt-sensitive rats, e.g., intimal and medial hyperplasia, with infiltration of inflammatory cells, and significant amelioration of the glomerular sclerotic changes. In contrast, interferon gamma affected neither blood pressure nor renal functions in spontaneously hypertensive rats. These data indicate that interferon gamma ameliorates the development of hypertension and vascular and renal injuries in Dahl salt-sensitive rats. The resolution of vascular and renal injuries contributes, in part, to the antihypertensive action of interferon gamma. (Hypertension 1992;19:804–808)

KEY WORDS • interferon type II • renal function • kidney • Dahl rats • spontaneously hypertensive rats

Evidence has been provided suggesting that there is an association between hypertension and immune dysfunction in humans and in experimental hypertensive animals.1–2 The mechanism of this interrelation is not fully understood. However, since White and Grollman3 reported the implications of autoimmunity in hypertension after renal infarct, an increasing amount of evidence has indicated that suppressor T cell dysfunctions and subsequent hyperimmunoglobulinemia or an increase in autoantibodies cause interferon gamma is derived from mature T lymphocytes, exhibited antihypertensive effects when administered in hypertensive animals.9,10 In particular, because interferon gamma is derived from mature T lymphocytes and possesses potent immunological actions,11 its administration would be expected to make up for the T cell dysfunction in hypertensive animals and thereby may exert some influence on the immunological process in the progression of vascular and renal injuries.

Most of these studies have been performed using SHR rats or rat models for secondary hypertension. However, there are few data available as to the role of the immune system in Dahl salt-sensitive (DS) rats, a genetic model for salt-induced hypertension. It has been reported that DS rats are more vulnerable to vascular injuries, e.g., intimal and medial hyperplasia and thrombotic formation with periarterial massive infiltration of inflammatory cells, or renal damage.12,13 These pathophysiological properties strongly suggest the role of the immune system in the genesis of hypertension in DS rats. Thus, in the present study, to test the hypothesis that immune dysfunction contributes to the development of hypertension in DS rats through vulnerability to vascular and renal injuries, we investigated the effects of interferon gamma on blood pressure elevation and examined the structural and functional alterations in the vascular wall and kidney when DS rats on a high salt diet were chronically treated with interferon gamma for 10 weeks.
Methods

Male 6-week-old DS (n=10) and Dahl salt-resistant (DR, n=10) rats were obtained from Brookhaven National Laboratories, Upton, N.Y., and bred by Eisai Co., Ltd., Tokyo. The rats were fed a high salt diet containing 4% NaCl (w/wt). Five DS and five DR rats were subcutaneously injected with recombinant murine interferon gamma (5x10^4 units/kg body wt; Toray Industries, Kanagawa, Japan) dissolved in 100 μl isotonic saline once a week, and the other rats were injected with 100 μl isotonic saline alone in the same procedure. The murine interferon gamma was produced in Chinese hamster ovary cells and purified by affinity chromatography. The purity was more than 99%, and the specific activity was 4.6x10^8 units/mg protein when measured by the standard cytopathic effect reduction assay. The first interferon gamma injection was given simultaneously with the start of the high salt diet. Systolic blood pressure was measured by the tail-cuff method every week around 2 PM before interferon gamma was injected. After 4 and 10 weeks, 24-hour urine was collected, and the rats were killed under pentobarbital anesthesia (30 mg/kg body wt i.p.). Blood samples were drawn from the inferior vena cava. Wet tissue weight of the heart and descending thoracic aorta was measured, and the kidney was obtained for histological investigation.

The kidney was fixed with 10% formalin solution, embedded in paraffin, and 2-μm sections were stained with hematoxylin and eosin and periodic acid-Schiff. The glomerular, vascular, and tubular lesions were evaluated by light microscopy using semiquantitative scoring methods. Briefly, severity of the lesions was graded from 0 to 4, and in the case of glomeruli, the glomerular sclerosing score was calculated by summing the products of the severity score and the percentage of glomeruli displaying the same degree of severity.

Urinary protein concentration was measured using a protein assay kit (Bio-Rad Laboratories, Richmond, Calif.). N-Acetyl-β-D-glucosaminidase (NAG) activity in urine was measured as an indicator of tubular injury using sodium-cresol-sulphonphthaleinyl N-acetyl-β-D-glucosaminide as its substrate (NAG assay kit, Shionogi Pharmaceutical Co., Osaka, Japan). Urinary and plasma levels of creatinine were measured with a creatinine autoanalyzer (Beckman Instruments Japan, Tokyo).

Male 6-week-old SHRs (n=25) and normotensive control Wistar-Kyoto (WKY) rats (n=14; Tokyo University breeding colony) were fed a regular laboratory chow, and interferon gamma (5x10^4 or 5x10^5 units/kg body wt) was administered in the same manner as in the Dahl rat experiment. After 10 weeks, the rats were killed and examined with the same procedure described in the Dahl rat study.

Values are expressed as mean±SEM. Differences were analyzed by the two-tailed Student’s t test and χ² test for independence.

Results

Figure 1 illustrates the alterations in systolic blood pressure over the therapeutic period by interferon gamma in DS rats (top panel) and in SHRs (bottom panel). In DS rats treated with interferon gamma, blood pressure declined 3 weeks after treatment was started, and the difference from the untreated group became greater in a time-dependent manner, being statistically significant in 9 weeks and thereafter. Interferon gamma did not influence blood pressure in normotensive DR rats. In contrast to DS rats, the development of spontaneous hypertension in SHRs was not influenced by the same dose (5x10^4 units/kg body wt) of interferon gamma. Moreover, we found that the elevation of blood pressure in SHRs was not affected at all even by a 10 times higher dose of interferon gamma (197±3 mm Hg for untreated SHRs versus 196±3 mm Hg for treated SHRs at the 10th week of the therapeutic period).

Table 1 summarizes the results of various measurements at the end of the therapeutic period. The body weight of DS rats was greater in the interferon gamma-treated group than in the untreated control group. Interferon gamma improved the plasma creatinine level and increased the creatinine clearance rate of DS rats. Urinary excretion of protein and NAG was higher in control DS rats than in control DR rats. However, in DS rats, the long-term interferon gamma treatment significantly reduced the urinary excretion of protein by 38% and NAG by 22%. More intriguing, the reductions in urinary protein and NAG excretion in DS rats were significant even at the 4-week stage when blood pressure was not different between the interferon gamma and control groups (31±5 versus 47±3 mg/day/100 g body wt for urinary protein excretion, p<0.05; 52±5 versus 74±8 milliunits/day/100 g body wt for urinary NAG excretion, p<0.05). On the other hand, these parameters of renal functions and renal injuries were not affected by interferon gamma treatment in DR rats.
Both cardiac weight and aortic thickness did not differ significantly between control and interferon gamma groups in DS or DR rats. In contrast to the values in DS rats, the long-term treatment with interferon gamma did not alter the various parameters indicating renal functions in DR rats, as shown in the right two columns of Table 1.

As indicated in the bottom row of Table 1, the glomerular sclerosing score was much higher in untreated DS rats than in untreated control DR rats (273±13 versus 81±6, p<0.001). Long-term interferon gamma treatment significantly lowered the sclerosing score by 22% in DS rats (213±22 versus 273±13, p<0.05). On the other hand, the glomerular sclerosing score in the untreated SHRs with established hypertension was almost equal to the score of normotensive control WKY rats (45±5 versus 39±6, p>0.5). In such a case, interferon gamma did not influence the regression of the sclerosis (42±7 for treated SHRs, p>0.7; 38±6 for treated WKY rats, p>0.9).

Figure 2 shows the representative microscopic appearance of the intrarenal arteries in Dahl rats (panels a–d) and in SHRs and WKY rats (panels e–h). In untreated DS rats, four of five kidneys exhibited one or more radiating arteries displaying intimal and medial hyperplasia, with destruction of the internal elastic layers, and the periarterial infiltration of inflammatory cells, indicating a malignant phase of hypertension (panel a). These lesions were almost completely resolved by the long-term interferon gamma treatment (zero of five kidneys; χ²=6.02, p<0.05) (panel b). There were no significant histological alterations in the arteries of the untreated and treated DR rats. More interestingly, the arteries from SHRs displayed almost normal appearance even when they were in the established hypertensive state, as indicated in panel e. Interferon gamma did not influence such normal arteries from SHRs, shown in panel f.

Discussion

In the present study, although both treated and untreated DS rats developed hypertension with a high salt diet, interferon gamma treatment attenuated further progression of salt-induced hypertension. Moreover, it was also shown that this blood pressure reduction was accompanied by structural and functional improvement in the arterial walls and kidneys. The cause of this antihypertensive effect and its relation to the improvement of renal lesions is not clear. However, because the improvement of the renal functions, as indicated by the decrease in urinary excretion of protein and NAG, preceded the decrease in blood pressure, it seems highly possible that the resolution of the morphological alterations and the improvement of renal functions are primary to the decrease in blood pressure in DS rats.

In this context, we showed that neither this dose nor a 10 times higher dose of interferon gamma was able to ameliorate the development of hypertension in SHRs. It is well documented that SHRs are resistant to vascular and renal damage in hypertension. In the present study, in fact, we found that the vascular walls had an almost normal appearance, and renal sclerosis was slight in untreated SHRs. The failure of interferon gamma to decrease blood pressure in SHRs conceivably can be attributed to the slightness of the vascular and renal injuries induced by hypertension. Such a possibility seems intriguing; however, we cannot make conclusive remarks until we investigate interferon gamma effects on SHRs with periarteritis, such as stroke-prone SHRs.

A few speculations can be offered as to the mechanism of the vascular and renal protection by interferon gamma. First, interferon gamma has been shown to inhibit the proliferation of vascular smooth muscle cells and endothelial cells. Moreover, interferon gamma also inhibits the gene expression of platelet-derived growth factor and interleukin-1, both of which are promoters of arteriosclerosis. These direct effects of interferon gamma on vascular cells could bring about the regression of hypertensive vascular lesions, thereby leading to the blood pressure reduction in DS rats. Second, it has been found that deposition of immunoglobulins or infiltration of mononuclear cells is seen in...
FIGURE 2. Light micrographs of intrarenal arterial injuries. Panels a–d: Dahl rats. Medial hyperplasia, hypercellularity of intima, and destruction of internal elastic layer were marked in the radiating artery from untreated Dahl salt-sensitive rats on a high salt diet (wide arrow in panel a). These alterations were accompanied by narrowing of the lumen and infiltration of the mononucleic cells in the periarterial spaces (small arrow in panel a). It should be noted that a much smaller artery displayed necrosis of smooth muscle cells (double-stranded arrow in panel a). Such vascular injuries were not observed in the interferon gamma–treated Dahl salt-sensitive rats (panel b). Radiating arteries and smaller arteries from untreated (double-stranded arrow in panel c) and treated (panel d) Dahl salt-resistant rats showed almost normal appearance. Panels e–h: Spontaneously hypertensive rats (SHRs) and Wistar-Kyoto (WKY) rats. There were no apparent changes in arterial appearance from untreated (panel e) and interferon gamma–treated (panel f) SHRs and from untreated (panel g) and treated (panel h) WKY rats. Periodic acid-Schiff stain, ×300.

the renal arterioles of hypertensive humans or animals, particularly in salt-induced hypertension and malignant hypertension.\textsuperscript{15,17,18} Therefore, it seems possible that modulation of the immune system by interferon gamma resulted in alleviation of the immunological process of vascular and renal injuries in salt-loaded DS rats.

Recently, Tuttle and Boppana\textsuperscript{9} reported that interleukin-2, a cytokine that stimulates T cell proliferation and differentiation, completely prevented the development of hypertension in SHRs. They also reported that interferon gamma exhibited a similar antihypertensive effect in SHRs.\textsuperscript{10} However, other investigators could not
confirm the antihypertensive effect of interleukin-2 in SHRs, even when a larger dose was given.\textsuperscript{19,20} Moreover, the present study on the effects of interferon gamma in SHRs was inconsistent with the data reported by Tuttle and Boppana. They showed that 500 units/kg body wt of interferon gamma produced permanent correction of the spontaneous hypertension. In the present study, we used a 100 times higher dose than Tuttle and Boppana, and the administration was repeated every week for 10 weeks to clarify whether this cytokine has any antihypertensive effect. However, our dose is still lower than the dose generally used in cancer therapy. The reason for the discrepancy between the two studies is not certain. We used murine recombinant interferon gamma. However, because a considerable cross-species activity is found between rat and mouse, this difference could not explain the inconsistent interferon gamma effect.\textsuperscript{14}

Although we administered the same dose of interferon gamma in the same manner, it may be possible that the pharmacokinetics of interferon gamma injected subcutaneously are different among the different rat strains, and this may have contributed to the absence of antihypertensive effects by interferon gamma in SHRs. This possibility cannot be ruled out based on our results, because we did not actually measure the pharmacokinetics of interferon gamma. However, considering that even a 10 times higher dose failed to alter the blood pressure of SHRs, this possibility is not likely to explain the marked difference in antihypertensive effects of interferon gamma between DS rats and SHRs.

Finally, we clearly demonstrated in this study that interferon gamma ameliorated the development of salt-induced hypertension and improved the structural and functional alterations in the vascular walls and kidneys of DS rats. Because interferon gamma was recently introduced in clinical use, we do not have much data as to its physiological effects on the cardiovascular system in humans. The data from the present study indicate that it is of great interest to investigate whether interferon gamma is capable of ameliorating high blood pressure and protecting the renal functions in essential hypertension. The ultimate goal of antihypertensive treatment is to protect patients against damage to target organs. Therefore, if this cytokine really attenuates vascular and organ injuries in humans, it would give us a new prospect for antihypertensive treatment.

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