pX Gene Causes Hypercholesterolemia in Hypercholesterolemia-Resistant BALB/c Mice

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To investigate the high incidence of atherosclerosis in the patients affected with rheumatoid arthritis, we examined the effect of feeding a cholesterol-enriched diet on the development of hypercholesterolemia in pX transgenic mice, which spontaneously develop chronic inflammatory arthritis. Cholesterol feeding to pX transgenic mice induced a striking elevation in serum total cholesterol (ca. 500 mg/dl) compared with their littermates, BALB/c mice used as controls. The pX transgenic mice exhibited elevated mRNA levels of ACAT1, and ABCG5 in the small intestine compared with their littermates, and furthermore, apoA1, ABCA1, ABCG5, ACAT1, and ACAT2 mRNAs were induced more easily by a cholesterol-enriched diet in pX transgenic mice than their littermates. As ACAT1 mRNA in the small intestine is known not to be induced by feeding a cholesterol-enriched diet, a possibility was inferred that interferon-gamma induced by Tax, a pX gene product, might play an important role in the induction of ACAT1 mRNA and the following hypercholesterolemia. These findings suggest that pX gene plays an important role in inducing hypercholesterolemia in BALB/c mice, which are genetically less susceptible to hypercholesterolemia and atherosclerosis and that RA patients carrying HTLV-1 virus have a predilection for hypercholesterolemia, a main risk factor for cardiovascular diseases.

Key words hypercholesterolemia; pX transgenic mouse; rheumatoid arthritis; acyl-CoA:cholesterol acyltransferase; ABC transporter

Recent accumulating evidence suggests that inflammatory processes mediate the initiation and progression of the atherosclerotic lesion, as well as contributing definitively to the development of acute ischemic syndromes. Recognition of the key role played by inflammation in atherogenesis augments the interest in cardiovascular disease among patients with rheumatoid arthritis (RA), a chronic inflammatory disease characterized by acute arthritis affecting several joints. Several key studies have been published during the past 4 years that exhibit evidence that RA patients have reduced life expectancy due to an increase in cardiovascular and cerebrovascular disease secondary to atherosclerosis. Non-invasive measurement of coronary artery lesions with ultrasonography also shows that carotid artery intimal-medial thickness is increased in RA patients. In addition, etiological studies indicate that RA is associated with inflammation-induced vascular endothelial dysfunction, leading, ultimately, to atherosclerosis. On the other hand, it is reported that the ratios of total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol are significantly higher in patients with untreated active RA than in controls. It became evident that patients with active RA have altered lipid profiles, which are atherogenic. Atheroma is thought to be a cholesteryl ester storage disease with a disorder of efflux of atherogenic lipids from the vessel wall. In fact, oxidized low density lipoprotein (LDL) and lysophosphatidyl choline produced in the subendothelium play a critical role in activation or injury of endothelial cells, resulting in the development of atherosclerosis. Hypercholesterolemia is a traditional risk factor and inflammation is thought to be an independent risk factor. Several animal studies strongly indicated that inflammation is not a sufficient condition for the development of atherosclerosis because atherosclerosis is not induced by immunization with bacillus Calmette-Guerin vaccine or by intravenous injection of endotoxin without a cholesterol-enriched diet. Chronic inflammation may act synergistically with traditional risk factors for cardiovascular disease and may be intimately linked with the initiation and acceleration of coronary heart disease in RA patients. Therefore, it is worthwhile elucidating the relationship between inflammation and traditional cardiovascular risk factors in order to extensively understand high incidence of atherosclerosis in RA patients.

A high incidence of chronic arthritis has been reported in patients with the human T-cell leukemia virus type I (HTLV-I)-associated myelopathy. To elucidate the pathological roles of HTLV-I in RA, pX transgenic mice which carry an HTLV-I env-pX region under its own long terminal repeat, were produced and found to develop chronic inflammatory arthropathy. Tax protein, encoded by pX gene, is responsible for the development of arthropathy in the transgenic mice. Tax not only regulates HTLV-I gene expression but also stimulates the transcription of several cellular genes including activating transcription factors/CRE-binding proteins (CREB), NF-κB/1B complex, p67SRF, and AP-1. Especially, NF-κB, which is highly activated by Tax, increases the resistance of synovial cells against several apoptotic stimuli, resulting in increased production of cytokines and cell proliferation. In addition, the complex formation of Tax with CREB-binding protein (CBP) enhances the expression of a variety of target genes related to cellular activation and growth, leading to the excess proliferation of synoviocytes and the synovitis. The pathology of the lesions in pX transgenic mice closely resembles that of RA in humans, indicating marked synovial and periarticular inflammation with articular erosion caused by invasion of granulation tissue. Thus, pX transgenic mice provide a useful animal model of spontaneously developed arthritis for RA in humans. The present study was, therefore, designed to investigate whether or not the transduction of pX gene into BALB/c mice, which are known to be less susceptible to hypercholesterolemia and atherosclerosis, affects cholesterol metabo-
lism.

We report here that the feeding of a cholesterol-enriched diet to pX transgenic mice induces a striking elevation in serum total cholesterol and that some genes concerning cholesterol absorption or secretion may be under the regulation by pX gene.

MATERIALS AND METHODS

Animals The pX transgenic mice carrying an HTLV-1 env-pX region were kindly provided by Dr. Iwakura of University of Tokyo. Male pX transgenic mice were bred with female BALB/c mice (Charles River Japan, Co. Ltd., Tokyo, Japan). The offspring were then used after determining the presence of the pX gene. The transgene was detected by PCR (primers used are described in Table 1) using DNA prepared from mouse tails. Littermates (BALB/c mice) were used as controls. All mice were housed in a temperature-controlled room (at 23 ± 1°C) with lighting from 6 a.m. to 6 p.m. under specific-pathogen-free conditions. They were fed a sterilized commercial diet with a low protein content (Nippon Crea Co., Ltd., Shizuoka, Japan) and given water ad libitum at the Laboratory Animal Center of Nagoya City University. The pX transgenic mice were weaned at 4 weeks of age and fed a commercial chow until they were started on a cholesterol-enriched diet. All animal procedures were approved by the Institutional Animal Care and Use Committee of Nagoya City University.

Diets The pX transgenic mice and their littermates matched for body weight at 17 weeks of age were assigned to one of two groups (n=4 to 6). Both groups of mice were maintained on a cholesterol-enriched diet for 7 weeks. The cholesterol-enriched diet used in the present study consisted of 1.25% cholesterol, 20% milk casein, 50% sucrose, 15% coconut milk, 4.95% crystallized cellulose, 1% corn oil, 5% mineral mixture, 1% vitamin mixture, 1% choline chloride and 0.3% methionine (Oriental Yeast Co., Ltd., Tokyo, Japan). The control diet contained 1.25% of additional sucrose in place of cholesterol. ApoE-deficient mice were fed a commercial chow (CE-2; Nippon Crea Co., Ltd., Shizuoka, Japan).

Analysis of Serum Lipids Following 16 h of fasting, mice were bled through retro-orbital veins under diethylester anesthesia and the serum was extracted. Serum total cholesterol and triglycerides were measured using an enzymatic sorption efficiency. The present study was, therefore, designed to investigate the effect of cholesterol feeding on the development of hypercholesterolemia in BALB/c mice with pX transgene, pX transgenic mice, which spontaneously develop chronic inflammatory arthropathy accompanying focal inflammation and an elevated immune response. The pX transgenic mice and their littermates, BALB/c mice, were maintained on a cholesterol-enriched diet for 7 weeks. The body weights of the pX transgenic mice or their littermates maintained on a cholesterol-enriched diet group or a control diet group, did not vary significantly (data not shown). However, serum total cholesterol levels in the pX transgenic mice were strikingly increased after starting the feeding of a cholesterol-enriched diet for 7 weeks (Fig. 1). In contrast, serum total cholesterol levels in the littermates did not increase compared to the pX transgenic mice. Serum triglyceride levels at the end of the experiment were not significantly different when comparing pX transgenic mice and their littermates, nor between the two diet groups (data not shown). When the lipoprotein profiles were examined using FPLC, very low density lipoprotein (VLDL) was markedly increased in the...
pX transgenic mice fed a cholesterol-enriched diet (Fig. 2), whereas high density lipoprotein (HDL) was essentially identical in the two groups of mice. Then, to elucidate the mechanism underlying the marked increase in serum total cholesterol in the pX transgenic mice resulting from the cholesterol-enriched diet, mRNA expressions of apoproteins and enzymes concerning absorption and secretion of cholesterol in the small intestine and liver were determined by semi-quantitative RT-PCR. First, mRNA levels in the small intestine of the pX transgenic mice and their littermates fed a control diet were determined as shown in Fig. 3. Interestingly, mRNA levels of acyl-CoA:cholesterol acyltransferase (ACAT) 1 and ATP binding cassette (ABC) transporters ABCG5 and ABCG8 were markedly increased by cholesterol-enriched diet both in the pX transgenic mice and their littermates (Fig. 3). In contrast, apoA1 and ACAT2 mRNAs did not vary between the two groups of mice, and ABCA1 mRNA was hardly detected in the small intestine. On the other hand, these mRNAs were similarly expressed in the livers of both groups (data not shown). When the effect of cholesterol-enriched diet on these mRNAs in the small intestine was assessed, it was found that the mRNAs of apoA1, ABCA1, ABCG5, ACAT1, and ACAT2 were markedly increased by cholesterol-enriched diet in the pX transgenic mice. Similar effects were observed in the ABCA1, ABCG5, ACAT1, and ACAT2 mRNAs of the littermates, but to a lesser extent when compared to the pX transgenic mice. However, the effect of a cholesterol-enriched diet on apoA1 was negligible in the littermates. These mRNAs in the liver were not influenced by feeding of a cholesterol-enriched diet both in the pX transgenic mice and their littermates (data not shown).

DISCUSSION

Intestinal cholesterol absorption is reported to be influenced by genetic factors at the enterocyte level, as demonstrated by significant differences in cholesterol absorption between different mouse strains, for example, between BALB/c and C57BL/6 mice.27) In the present study, pX transgenic mice, BALB/c mice with pX transgene, fed a cholesterol-enriched diet exhibited markedly higher serum total cholesterol levels comparable to apoE-deficient mice with a genetic background of C57BL/6. However, the pX transgenic mice fed a control diet did not show the increase in serum total cholesterol levels. This result suggests that pX transgenic mice could have genetic variations in cholesterol absorption at the small intestine and that pX gene would not influence the uptake of lipoproteins in the liver by lipoprotein receptors.

The molecular mechanism underlying cholesterol absorption in the small intestine is not fully understood.26) Dietary cholesterol first enters the enterocytes of the duodenum and the jejunum either by passive diffusion or by an unidentified protein-mediated process.28) Cholesterol is then esterified by ACAT2 to form cholesteryl ester, which is secreted from the basolateral surfaces of the enterocytes as part of chylomicrons. On the other hand, ABCG5 and ABCG8 restrict the amount of cholesterol available for esterification by ACAT2, stimulate efflux of cholesterol into intestinal lumen, and limit the absorption of dietary cholesterol.29,30) When the mRNA levels of the transporters or enzymes concerning cholesterol absorption at the small intestine are elevated in the small intestine of the transgenic mice, BALB/c mice, were maintained on a cholesterol-enriched diet or control diet for 7 weeks. Open circle: pX transgenic mice on control diet, closed circle: pX transgenic mice on cholesterol-enriched diet, open triangle: BALB/c mice on control diet, closed triangle: BALB/c mice on cholesterol-enriched diet. Each value represents mean±S.E. of 4 to 6 mice. ∗p<0.05, ∗∗p<0.01 versus pX transgenic mice on control diet, †p<0.05, ††p<0.01 versus BALB/c mice on control diet.
absorption or secretion in the small intestine were evaluated, ACAT1 mRNA was found to be elevated in the pX transgenic mice compared with their littermates and further increased markedly by feeding a cholesterol-enriched diet. In mammals, two ACAT isozymes have been identified, ACAT1 and ACAT2.31—34) The expression of ACAT1 is ubiquitous, whereas the expression of ACAT2 is restricted to the liver and small intestine.35) In general, ACAT1 functions in intracellular cholesterol homeostasis in many tissues, whereas ACAT2 is involved in the synthesis of cholesterol esters destined for secretion in the core of apob-containing lipoprotein particles in the liver and intestine. In the small intestine, ACAT2 was mostly localized in the apical domain of the mucosal cells at a relatively high concentration, whereas ACAT1 was scattered throughout the mucosal cell and further its expression was very low.36) Based on these kinds of evidence, ACAT2 is thought to be the major cholesterol-esterification enzyme in the small intestine.35,37) ACAT2-deficient mice actually show resistance to diet-induced hypercholesterolemia and cholesterol-gallstone formation,38) and little or no reduction was observed when ACAT1 was deficient.39)

In our study, ACAT1 mRNA levels were very low or undetectable in the small intestines of BALB/c mice regardless of a feeding of cholesterol-enriched diet, which coincided with previous studies.40) However, ACAT1 mRNA levels in the small intestines of pX transgenic mice were elevated compared with those of their littermates BALB/c mice and markedly increased by feeding a cholesterol-enriched diet. This result suggests that ACAT1 may influence intestinal cholesterol absorption and may be responsible for the following hypercholesterolemia, if the expression of ACAT1 becomes comparable in amount to that of ACAT2. ACAT1 expression is known to be regulated by at least two mechanisms: transcriptional and post-transcriptional regulation. ACAT1 mRNA in the liver increased 2—3 fold in mice fed an atherogenic diet, while ACAT1 mRNA in the small intestine showed no change or a slight decrease by feeding an atherogenic diet.40) Since cholesterol-feeding itself is not capable of increasing ACAT1 mRNA levels in the small intestine,40) the possibility exists that the increase in ACAT1 mRNA and synergistic induction of ACAT1 mRNA by a cholesterol-feeding in the small intestines of pX transgenic mice is caused through pX gene-involved mechanism. Intracellular expression of Tax encoded by pX gene is associated with rapid up-regulation of interferon-γ (IFN-γ).41) In the joints of the patients with RA or arthritic joints of pX transgenic mice, various cytokines including IFN-γ, IL-1α, IL-1β, IL-6, TNF-α, TGF-β1, and IL-2 have been also found to be overexpressed.24) IFN-γ can increase the cellular cholesterol ester content by decreasing cholesterol efflux through pathways that include the upregulation of ACAT1.42) These studies may indicate that the increase in ACAT1 mRNA of pX transgenic mice appears to be related with Tax expression, although the studies concerning atherogenic effect of IFN-γ are still confined to macrophage or macrophage-like cell lines. In addition, the mechanism underlying the synergistic induction of ACAT1 mRNA by a cholesterol-feeding remains to be clarified.

In the present study, ABCG5 mRNA levels was also increased in pX transgenic mice. ABCA1 and ABCG1 are ABC transporters that are involved in the movement of cholesterol from cells to HDL and its apolipoproteins. ABCG5 and ABCG8 regulate cholesterol absorption from the small intestine and cholesterol excretion from the liver into the bile.29,30) The expressions of these transporters are known to be activated by the heterodimers of liver X receptor and retinoid X receptor.43) In addition, TGF-β a cytokine induced by Tax, can increase cholesterol efflux by enhancing ABCA1 expression, or by antagonizing the inhibitory effect of IFN-γ on ABCA1 induction,44) resulting in preventing foam cell formation. Taken together, like ABCA1 mRNA, ABCG5 mRNA levels may be increased by TGF-β.

Further, apoA1, ABCA1, and ABCG5 mRNAs could be more easily induced by cholesterol-feeding in the pX transgenic mice than in their littermates, like ACAT1 and ACAT2 mRNA. Based on the evidence that ACAT1 mRNA in the small intestine cannot be induced by cholesterol-feeding and that apoA1, ABCA1, and ABCG5 mRNAs are induced by cholesterol-feeding, cholesterol increased in the small intestine following ACAT1 induction by IFN-γ might cause a significant elevation of apoA1, ABCA1, and ABCG5 mRNA levels. However, although ABCG5 and ABCG8 are known to reduce intestinal cholesterol absorption and increase biliary cholesterol secretion, they do not significantly affect plasma cholesterol levels. Because the elevated clearance of cholesterol from the body is balanced by an increase in hepatic cholesterol synthesis. In addition, ABCA1 may also serve to efflux cholesterol back into the intestinal lumen for elimination,45,46) although the contribution of ABCA1 to the suppression of cholesterol absorption in the small intestine is much smaller than that of ABCG5 and ABCG8. Therefore, the elevated ABCG5, and ABCA1 mRNAs do not seem to significantly antagonize the induction of hypercholesterolemia in the pX transgenic mouse.

RA patients exhibit higher ratios of total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol compared with the values observed in controls,5) although a contradictory result was reported by one group.47) Patients with RA caused by HTLV-1 infection may, in particular, have a high risk of hypercholesterolemia induced by the intake of a cholesterol-enriched diet.

In conclusion, the findings in the present study suggest that pX gene is likely to influence the expression of genes involved in intestinal cholesterol absorption, resulting in striking induction of hypercholesterolemia in the BALB/c mouse with a relatively low intestinal cholesterol absorption efficiency. However, the detailed mechanisms by which mRNAs of ACATs and ABC transporters are elevated in pX transgenic mice and are more easily induced than in their control littermates remain to be clarified via future study.

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