Differential Effects of Interleukin-1 Receptor Antagonist and Tumor Necrosis Factor Binding Protein on Fatty-Streak Formation in Apolipoprotein E–Deficient Mice

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Background—The cytokines interleukin 1 (IL-1) and tumor necrosis factor (TNF) are secreted by the different cell populations of the vascular wall and have been suggested to promote atherosclerosis.

Methods and Results—Their respective roles in fatty-streak formation in apolipoprotein E–deficient mice were investigated by use of IL-1 receptor antagonist and TNF binding protein. Estradiol-17β was used as a positive control. Blocking TNF seemed to be active in female animals but not in males. IL-1 receptor antagonist was as effective as or more effective than estradiol in both sexes.

Conclusions—IL-1 plays a crucial role in the initial step of the atherosclerotic process in this animal model, and blocking the activity of this cytokine should be considered as a therapeutic possibility. (Circulation. 1998;97:242-244.)

Key Words: atherosclerosis • apolipoproteins • interleukins • tumor necrosis factor

It is known that IL-1α and -1β and TNF-α and -β are secreted in the vascular wall by endothelial and smooth muscle cells as well as by monocytes/macrophages. These cytokines have been shown to increase permeability of the endothelial cell barrier, induce the expression of surface leukocyte adhesion molecules, and enhance the production of other cytokines and growth factors, such as IL-6 and macrophage colony-stimulating factor, all such activities being considered to promote atherosclerosis. The objective of the present study was therefore to clarify the role of IL-1 and TNF in the initial steps of the atherosclerotic process, ie, fatty-streak formation, using apo E KO mice as an animal model of atherosclerosis and human IL-1ra and TNFbp as the specific cytokine antagonists. IL-1ra is a recombinant 17-kD protein, which binds to IL-1 receptors and competes with both IL-1α and IL-1β without detectable IL-1 agonistic effects. TNFbp is a specific TNF inhibitor consisting of two molecules of the extracellular domain of the human type 1 TNF receptor added to both ends of a molecule of polyethylene glycol. TNFbp binds with equal affinity to TNF-α and TNF-β. E2 treatment was used as a positive control, because we and others have shown that this hormone prevents fatty-streak formation in the apo E KO mouse animal model. The data obtained showed that TNFbp was active in female animals but not in males. Like E2, IL-1ra was active in both sexes, suggesting that IL-1 plays a crucial role in the initial step of the atherosclerotic process in this animal model.

Methods

Study Protocol

Apo E KO mice, originally obtained from the Jackson Laboratory, Bar Harbor, Me (sixth generation of backcross from 129/B6 F1 heterozygous to C57BL/6), were housed as previously described and fed normal laboratory mouse chow containing 4.3% fat and 0.02% cholesterol. Four-week-old animals were gonadectomized under general anesthesia. At 2 months of age, these animals were given 0.2 mg 60-day time-release E2 pellets (Innovative Research of America), a dose that was found to exert a maximal effect on fatty-streak formation, or placebo pellets associated with human IL-1ra treatment (25 mg · kg body wt -1 · d -1), human TNFbp (1 mg · kg body wt -1 · d -1), or BSA (25 mg · kg body wt -1 · d -1). IL-1ra and TNFbp were kindly provided by Amgen Inc. IL-1ra was administered by Alzet 2004 osmotic minipumps (Alza Inc) implanted in a dorsal subcutaneous pocket. TNFbp or BSA was injected subcutaneously every other day. When mice were 2 months of age, to accelerate the atherosclerotic process, a pelletted “atherogenic” diet containing 16% (wt/wt) fat (primary cocoa butter), 1.16% cholesterol, and 0.5% cholic acid was also given to all animals until they were euthanized at 3 months old. TNFbp- or BSA-treated animals were killed 24 hours after the last subcutaneous injection. All experimental protocols were performed in accordance with the recommendations of the French Accreditation of Laboratory Animal Care.

Analytical Measurements

The conditions of lipid analysis, evaluation of serum hormone concentrations, and fatty-streak lesions have been described. Cellular composition was appreciated by immunohistochemistry using the monoclonal rat anti-mouse MOMA-2 antigen (SEROTEC) and the monoclonal mouse anti-α-actin immunoglobulin (DAKO SA).
Results

Effect of In Vivo Treatment With E2, IL-1ra, and TNFbp on General Status and Lipids in Apo E KO Mice

At the time of euthanasia, the body weights were lower in this series of experiments (Table 1) than in our previous study of animals of the same age. Administration of the atherogenic diet may explain this difference, which concerned control as well as treated animals. None of the mice exhibited side effects caused by administration of IL-1ra or TNFbp. Human IL-1ra and TNFbp were not detectable in the sera of E2- or BSA-treated mice (Table 1). The serum concentrations of IL-1ra were within the range of those reported previously by Kitazawa et al. but a log range higher for TNFbp concentrations measured 24 hours after the last injection. Moreover, a large scatter of the individual concentrations was observed, which probably reflected the emptying of the IL-1ra osmotic minipumps after 1 month of delivery. No clear explanation was apparent for TNFbp concentrations. In agreement with the data by Zhang et al., total serum cholesterol concentration reached very high levels (Table 1), which appeared in the VLDL-LDL size range on fractionation of the lipoproteins (data not shown). Also in agreement with the data of Bourassa et al. and our own data, E2 treatment reduced serum cholesterol levels, although this decrease was not significant in males (P = .074). Plasma cholesterol was unaffected by IL-1ra (P = .92 for females and P = .14 for males) and TNFbp (P = .33 and P = .47 for females and males, respectively) treatments.

Morphometric Evaluation of Fatty-Streak Formation

The extent of fatty-streak formation in the aortic sinus was examined. The average lesion size in castrated BSA-treated animals fed the atherogenic diet for 4 weeks was 10 times larger than in animals of the same age fed regular chow. Females had more extensive lesions than their male littermates (Tables 1 and 2; P < .03), and there was a significant interaction on the lesion size between sex and treatments (P = .038). Therefore, responses of female and male mice were analyzed separately. Fatty-streak area decreased significantly under E2 treatment in females (P < .002) but not in males (P = .088). The decrease was highly significant in IL-1ra-treated female and male animals (P < .001). The results were different for TNFbp-treated animals, in which a decrease was of borderline significance in females (P = .059) but insignificant in males (P > .99).

Histologically, in agreement with Zhang et al., the exacerbated progression of atherosclerotic lesions led to substantial arterial occlusion and increased complexity, with multilayered foam cells admixed with acellular lipid and necrotic cores and intense fibrous reaction. Immunohistochemical analysis showed that under all three treatments and despite the difference in lesion size, the cellular composition of the lesions, macrophages, and smooth muscle cells appeared similar to those of castrated BSA-treated animals (data not shown).

Discussion

The most important finding of this study was the demonstration that specific antagonism of IL-1 activity was as effective as or more effective than E2 in reducing fatty-streak formation in both male and female apo E KO mice, without evident interference with lipid metabolism. This suggests a crucial role of IL-1 during the initial step of the atherosclerotic process. Despite comparable serum concentrations, TNFbp was less effective in female animals and was inactive in males, suggesting that the potency of IL-1ra was superior to that of TNFbp. These data should be interpreted with caution, because of the respective sizes and structures of the two antagonists or the

Table 1. Effects of BSA, E2, IL-1ra, and TNFbp Treatment on Body Weight, Serum E2, Total Cholesterol, and Lesion Area in Castrated Female Mice

<table>
<thead>
<tr>
<th></th>
<th>BSA (n=7)</th>
<th>E2 (n=3)</th>
<th>IL-1ra (n=6)</th>
<th>TNFbp (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>17.1 ± 0.8</td>
<td>18.6 ± 0.7</td>
<td>17.8 ± 0.5</td>
<td>17.8 ± 0.9</td>
</tr>
<tr>
<td>E2, pg/mL</td>
<td>ND</td>
<td>220 ± 23</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Uterine weight, mg</td>
<td>&lt;20</td>
<td>120.4 ± 22.3</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>IL-1ra, μg/mL</td>
<td>ND</td>
<td>ND</td>
<td>2.2 ± 0.6</td>
<td>ND</td>
</tr>
<tr>
<td>TNFbp, μg/mL</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.33 ± 0.20</td>
</tr>
<tr>
<td>Total cholesterol, g/L</td>
<td>34.5 ± 2.4</td>
<td>23.6 ± 4.3*</td>
<td>34.2 ± 2.0</td>
<td>34.2 ± 2.1</td>
</tr>
<tr>
<td>Lesion area, μm²</td>
<td>247,801 ± 33,785</td>
<td>65,457 ± 14,669*</td>
<td>92,501 ± 12,081*</td>
<td>162,133 ± 19,457</td>
</tr>
</tbody>
</table>

*Statistically different from BSA-treated animals after logarithmic transformation.
possible development of antibodies to human TNFβp. Compared with our previous studies,21 the use of an athrogenic diet may have evidenced this sex difference in TNFβp effectiveness by revealing the female tendency to develop extensive lesions, which was not apparent with a chow diet. Current knowledge of plasma TNF concentrations20 or the function of TNF ligand and receptor families21 does not provide an explanation for this unexpected difference. Further studies are warranted, including time course and dose-response analysis, which could not be done in the present study because of shortage of the inhibitors, to determine the molecular and cellular mechanisms involved. The relevance of these observations in relation to sexually dimorphic immune responsiveness will also have to be clarified,25 but observations of accelerated fatty-streak development in mice deficient in p55 TNF receptor23 and future studies in TNF-, lymphotoxin-, or p55/p75 TNF receptor–deficient mice should take sex into consideration in the analysis of the data.

In conclusion, TNF in females and IL-1 in both sexes appear to play a crucial role in the constitution of fatty-streak lesions, the initial step of the atherosclerotic process, in the apolipoprotein E–deficient mouse model. Blocking the activity of these cytokines, especially IL-1, should now be considered as a therapeutic possibility.

Acknowledgments

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References

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