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Enhancing Effect of Dietary Cholesterol and Inhibitory Effect of Pravastatin on Allergic Pulmonary Inflammation

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Key Words
Cholesterol, dietary · Pulmonary allergic inflammation · Paravastatin · Eosinophil infiltration · Murine asthma model

Abstract
To elucidate the link between the intake of animal fat and asthma, a murine model was developed to examine the effect of dietary cholesterol on pulmonary allergic inflammation. Male C57BL6 mice were fed either a control diet or a diet supplemented with 2% cholesterol. Following sensitization and inhalation exposure to ovalbumin, the bronchoalveolar lavage fluid of mice in the cholesterol group contained higher numbers of eosinophils and elevated levels of IL-5, PGE_2, and MCP-1. In addition, dietary cholesterol also resulted in elevated production of IL-4 and IFN-γ by lymphocytes isolated from the lungs. These inflammatory indicators were all significantly correlated with serum cholesterol levels. In contrast to the effect of dietary cholesterol, adding pravastatin to the drinking water significantly reduced eosinophil infiltration and the levels of IL-5, PGE_2 and MCP-1 in lavage fluid. Although dietary cholesterol did not alter baseline IL-12 in the lungs, in mice challenged with ovalbumin the IL-12 levels were reduced in the cholesterol group and elevated significantly in the pravastatin group. The results suggest that dietary cholesterol might enhance pulmonary allergic inflammation, possibly involving both nonspecific inflammatory processes and lymphocyte activities.

Introduction

Epidemiological studies have suggested a link between the intake of animal fat and asthma symptoms in adolescents [6, 7]. To provide the association with biological relevance, we developed a murine asthma model to examine the effect of dietary cholesterol on allergic inflammation. Results showed that dietary cholesterol supplementation resulted in significant increase in the number of eosinophils and the level of interleukin-5 (IL-5) in bronchoalveolar lavage fluid (BALF) [23]. Because allergic inflammation is regulated by T-helper 2 cells (Th2), we needed to examine whether the cytokine mediator milieu in the lungs was altered by dietary cholesterol and affected lymphocyte activities. Some mediators play key roles in the regulation of T-cell development, for example, prosta-

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glandin E_2 (PGE_2) dose-dependently inhibited the ability of myeloid dendritic cells to produce interleukin-12 (IL-12), resulting in a type-2 polarized effector subset, and an environment with higher PGE_2 and/or lower IL-12 levels at the early stages of T-cell activation which may favor Th2 over T-helper 1 cells (Th1) [9]. The characteristics of antigen-presenting cells [19], such as the cytokine and chemokine receptor profile, may also determine the deviation in T-cell development [15, 21]. For instance, in addition to being a chemoattractant for memory T lymphocytes, monocyte chemoattractant protein-1 (MCP-1) also plays a role in immune deviation. Interleukin-4 (IL-4) production was enhanced when T cells were stimulated in the presence of MCP-1 [10], and MCP-1-deficient mice were unable to mount a Th2 response [4]. The expression of these mediators needs to be characterized in an animal model to elucidate the mechanism of dietary cholesterol-induced enhancement in allergic inflammation.

Anti-inflammatory agents are the mainstay of asthma treatment. Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase. In addition to the well-established uses for statins as serum lipid-lowering agents, their effect as immunomodulators was recently reported. Statins inhibit the expression of class-II major histocompatibility complex (MHC-II) induced by interferon-γ (IFN-γ) and suppress MHC-II-mediated T-cell activation [13]. Statin may also inhibit integrin-mediated T-cell adhesion and costimulation through its specific binding site on β_2-integrins [22]. Therefore, statins seem to be able to alleviate immunological pathologies. On this front, simvastatin was shown to suppress autoimmune arthritis in a murine model [14], but the effect of statins on allergic inflammation has not been investigated.

With the observed enhancing effect of dietary cholesterol on pulmonary eosinophilic inflammation, we sought to delineate the mechanism by assessing a range of important inflammatory mediators and lymphocyte functions. In addition, we tested the potential inhibitory effect of statins on allergic inflammation.

Materials and Methods

Animal Feeding, Sensitization, and Challenge

Weanling male C57BL/6 mice were obtained from in-house breeding at Taiwan National Laboratory Animal Center at 4 weeks of age and kept at the animal facility of Yang-Ming University. The animals were maintained at 22–24°C with a 12-hour dark/light cycle. The mice were fed either control diet containing 200 ppm cholesterol (Rodent Diet 5001; Lab Diet, Mo., USA) or diet supplemented with 2% (weight/weight) cholesterol (Sigma, St. Louis, Mo., USA). The mice were sensitized at the 9th week by intraperitoneal (i.p.) injection of 50 μg ovalbumin (OVA; Grade V; Sigma) with 2 mg alum. Fourteen days after sensitization, the mice were exposed to aerosols of saline or 2% OVA in saline, for 20 min/day for 3 consecutive days. The aerosols were generated by a DeVilbiss nebulizer driven by air compressor (PulmoAide, DeVilbiss, Somerset, Pa., USA). Bronchoalveolar lavage (BAL) was performed 24 h after the last aerosol inhalation. In the statin group, the mice were fed a control diet, and statin was supplied in drinking water from the 7th week; statin (Mevalotin, Sankyo, Japan) was dissolved in water at a concentration of 0.08 mg/ml, which gave a daily dose of 0.4 mg when water consumption was assumed to be 5 ml.

Bronchoalveolar Lavage

Twenty-four hours after the last aerosol inhalation, the animals were euthanized with sodium pentobarbital. BAL was performed by 5 washes of 0.6 ml prewarmed (37°C) phosphate-buffered saline (PBS). The first lavage fluid was centrifuged at 300 *g* for 10 min, and the supernatant was stored at −70°C. Cells in the first wash were combined with the remaining washes and counted. Cytospin slides were fixed and stained with the Hemacolor Stain Set (Merck, Germany), and at least 400 cells were counted for leukocyte differential counts.

Lymphocyte Isolation and Stimulation in vitro

After lavage, the chest was opened, and blood was drawn from the right ventricle, and the sera were kept at −20°C until analysis. The lungs were perfused with 5 ml PBS to remove blood cells, and then they were carefully removed from the chest. The left lobe of the lung was finely minced and homogenized in 0.5 ml PBS, centrifuged at 500 *g* for 10 min, and the supernatant was kept at −70°C. With the cannula in place, the right lobe was infused with 0.6 ml 0.2% (weight/volume) pronase (Sigma) in PBS, immersed in 3 ml PBS, and kept at 37°C in a water bath for 30 min. Loose cells were squeezed out of the lung with forceps and washed with RPMI (Irvine Scientific, Santa Ana, Calif., USA) supplemented with 10% fetal bovine serum (FBS). The cells were washed twice more with PBS and incubated with RPMI supplemented with 2% FBS for 2 h at 37°C and 5% CO_2. Afterwards, cells were carefully layered on Histopaque 1.083 (Sigma) and centrifuged at 300 *g* for 30 min. The viability of cells was >90% by trypan blue exclusion, and cytospin showed that the cells were composed of 60–70% lymphocytes, ~20% eosinophils, and ~10% macrophages. Mononuclear cells were washed twice with PBS and cultured in 24-well plates (flat-bottom; Costar, New York, N.Y., USA) with a density of 2.5×10^6 cells/ml in RPMI with 5% autologous sera. The cells were cultured in medium alone or stimulated with 10 ng/ml PMA plus 1 μg/ml ionomycin (both from Sigma) for 24 h. After incubation, the medium was aspirated, centrifuged at 300 *g* for 10 min, and kept at −70°C.

Serum Cholesterol

Total serum cholesterol concentration was measured by photometric determination method (cholesterol test kit; Merck). Briefly, cholesterol and its esters are released from lipoproteins by detergents and treated with cholesterol esterase. In the subsequent enzymatic oxidation by cholesterol oxidase, H_2O_2 is formed, which is converted into a quinonimine in a reaction with 4-aminoantipyrine and salicylic alcohol catalyzed by peroxidase and measured by optic density at 500 nm.

Table 1. Leukocyte numbers per mouse ($\times 10^4$ cells, mean ± SD) and differential count (mean percentages in parentheses) in bronchoalveolar lavage fluid of mice in 3 groups: control diet (control), diet with 2% cholesterol (cholesterol), and control diet with pravastatin in drinking water (pravastatin)

Diet	Inhalation	Total cells	Eosinophil	Neutrophil	Lymphocyte	Macrophage
Control	Saline (n = 12)	68 ± 31	0.05 ± 0.1 (0.04)	0.03 ± 0.08 (0.03)	1.8 ± 1.7 (2.25)	67 ± 30 (97.6)
	OVA (n = 20)	391 ± 197[a]	265 ± 174[a] (62.2)	25 ± 16[a] (7.0)	52 ± 22[a] (15.5)	49 ± 18 (15.5)
Cholesterol	Saline (n = 8)	80 ± 31	1.0 ± 2.6 (0.97)	0.1 ± 0.2 (0.1)	4.9 ± 8.8 (5.1)	74 ± 29 (93.7)
	OVA (n = 20)	605 ± 282[a,b]	442 ± 228[a,b] (71.4)	40 ± 22[a,b] (7.1)	67 ± 32[a] (11.3)	55 ± 35 (10.2)
Pravastatin	OVA (n = 12)	241 ± 155[c]	133 ± 132[c] (46.5)	27 ± 19 (8.3)	45 ± 25 (22.3)	42 ± 10 (22.9)

[a] Significantly higher than saline-exposed mice in the same diet groups ($p < 0.001$).
[b] Significantly higher than OVA-exposed mice of the control diet group ($p < 0.05$).
[c] Significantly lower than OVA-exposed mice of the control diet group ($p < 0.05$).

In vitro Culture

Several cell lines were tested in vitro to examine the effect of lipoprotein, including mouse macrophage (RAW 264.7), human monocyte (THP-1), and human airway epithelial cell (A549). The culture media were DMEM with 2% FBS (RAW 264.7), RPMI 1640 with 5% FBS (THP-1), and F12 with 2% FBS (A549). Native human plasma low-density lipoprotein (LDL; Sigma) or oxidized LDL (ox-LDL) was added to culture media. LDL was oxidized by short-wave-length ultraviolet irradiation [17]. The efficiency of oxidation was determined by LPO-586 (Bioxytech; OXIS Health Products, Portland, Oreg., USA); native LDL contained 0.093 nmol MDA/mg protein, and oxidized LDL contained 3.11 nmol MDA/mg protein.

Cytokine and Chemokine Measurement

All cytokines were quantified by ELISA kits from the following suppliers: IL-5 and MCP-1 by OptEIA ELISA kit (BD PharMingen, San Diego, Calif., USA); IL-12 (p70) by sandwich ELISA kit (Endogen, Woburn, Mass., USA); IFN-γ and IL-4 by Immunoassay Kit (BioSource International Inc, Camarillo, Calif., USA). PGE_2 was measured by competitive EIA (Prostaglandin E_2 EIA kit-Monoclonal; Cayman Chemical, Ann Arbor, Mich., USA). All materials were used according to the instructions of the manufacturers.

Statistical Analysis

Data for analysis were combined from several runs of experiments. Data distribution was tested for normality. If data of a variable fit normal distribution, analysis of variance was applied to compare difference between groups (e.g., body weight, serum cholesterol, and MCP-1 in lung tissue). For data that are not normally distributed, the Wilcoxon rank sum test was used. A difference with a p value of <0.05 was considered statistically significant. All statistical analyses were made using the Statistical Analysis System package (SAS 8.1, SAS Institute, Cary, N.C., USA).

Results

Effect of Cholesterol and Pravastatin on Serum Cholesterol Level and Body Weight

The serum cholesterol level was significantly different among the 3 diet treatment groups: 111.6 ± 17.0 mg/dl in control diet group (n = 32), 145.7 ± 24.3 mg/dl in cholesterol group (n = 28), and 81.8 ± 12.7 mg/dl in the pravastatin group (n = 12; $p < 0.001$). Compared with mice with saline inhalation, OVA challenge did not affect serum cholesterol. There was no significant difference in body weight among treatment groups.

Cholesterol and Statin Effects on Pulmonary Inflammation

All mice were sensitized intraperitoneally with OVA, and after 2 weeks were exposed to either saline or OVA aerosol. OVA inhalation caused a significant increase in eosinophils, neutrophils, and lymphocytes in the BALF, demonstrating the expected effect of antigen challenge. Mice fed the cholesterol diet had exaggerated responses to OVA inhalation, as shown by the higher numbers of eosinophils which were on average 2.68 times those of the control diet group (table 1). In contrast, eosinophil infiltration in mice of the pravastatin group was significantly diminished.

Table 2. PGE_2, IL-5, and MCP-1 concentrations (pg/ml) in BALF and IL-12 (pg/ml) in lung tissue of mice in 3 groups (mean ± SD, number of mice in parentheses): control diet (control), diet with 2% cholesterol (cholesterol), and control diet with pravastatin in drinking water (pravastatin)

	Inhalation	BALF PGE_2	BALF IL-5	BALF MCP-1	Homogenate IL-12
Control	Saline	348 ± 86 (n = 11)	ND (n = 5)	ND (n = 5)	1,476 ± 396 (n = 11)
	OVA	962 ± 407[a] (n = 18)	20.5 ± 13.5[a] (n = 10)	40.6 ± 34.8[a] (n = 15)	1,419 ± 476 (n = 14)
Cholesterol	Saline	324 ± 69 (n= 8)	ND (n = 5)	ND (n = 5)	1,419 ± 224 (n = 7)
	OVA	2,276 ± 1,280[a,b] (n = 11)	61.5 ± 48.5[a,b] (n = 9)	70.1 ± 48.1[a] (n = 14)	1,155 ± 278[c] (n = 15)
Pravastatin	OVA	523 ± 138[d] (n = 8)	6.2 ± 6.0[d] (n = 8)	16.4 ± 19.3[d] (n = 12)	2,236 ± 756[e] (n = 9)

ND = Not detectable.

[a] Significantly higher than saline-exposed mice in the same diet groups ($p < 0.05$).

[b] Significantly higher than OVA-exposed mice of the control diet group ($p < 0.05$).

[c] Significantly lower than saline-exposed mice in cholesterol diet group ($p < 0.05$).

[d] Significantly lower than OVA-exposed mice of the control diet and cholesterol diet groups ($p < 0.05$).

[e] Significantly higher than OVA-exposed mice of the control diet and cholesterol diet groups ($p < 0.05$).

Table 3. The correlation matrix of total cells, eosinophils, PGE_2, IL-5, and MCP-1 concentrations in BALF, and IL-12 concentration in lung tissue homogenate[1]

	BALF total cells	BALF eosinophils	BALF PGE_2	BALF IL-5	BALF MCP-1	Homogenate IL-12
BALF eosinophils	0.991 <0.0001					
BALF PGE_2	0.597 <0.0001	0.578 0.0002				
BALF IL-5	0.710 <0.0001	0.686 <0.0001	0.601 0.008			
BALF MCP-1	0.674 <0.0001	0.639 <0.0001	0.681 <0.0001	0.347 0.077		
Homogenate IL-12	−0.513 0.001	−0.501 0.001	−0.456 0.022	−0.522 0.011	−0.526 0.003	
Serum cholesterol	0.534 <0.0001	0.539 <0.0001	0.460 0.004	0.502 0.008	0.464 0.002	−0.633 <0.0001

[1] Pearson's correlation coefficient and p values.

PGE_2, IL-5, and MCP-1 in BALF Increased by Dietary Cholesterol and Decreased by Pravastatin

We measured in the BALF several mediators related to allergic inflammation, including PGE_2, IL-5, and MCP-1. In sensitized mice with saline inhalation, the PGE_2 levels in BALF were similar in control diet and cholesterol groups, whereas IL-5 and MCP-1 were not detectable (table 2). Corresponding to the magnitude of pulmonary inflammation, there was significant increase in PGE_2, IL-5, and MCP-1 in BALF following OVA challenge, and the increase was much more pronounced in the cholesterol group ($p < 0.05$ for PGE_2 and IL-5, $p = 0.07$ for MCP-1) (table 2). Also in accord with its inhibitory effect on the number of recruited inflammatory cells, pravastatin significantly reduced the levels of PGE_2, IL-5, and MCP-1 in BALF. As expected, there were high levels of correlation

between the number of inflammatory cells and concentrations of these mediators in BALF (table 3). Interestingly, each of the indicators of inflammation was also significantly correlated with serum cholesterol level (table 3).

IL-12 Levels in Lung Parenchyma

Besides PGE_2 and MCP-1, IL-12 also plays a crucial role in regulating naïve T-cell development. In mice fed the control diet, IL-12 concentrations were not different with saline- or OVA-challenge – that is, antigen challenge did not alter IL-12 expression. Unexpectedly, in mice in the cholesterol group, IL-12 levels were lower when challenged with OVA ($p = 0.16$), and this reduction was reversed by pravastatin ($p = 0.015$) (table 2).

IL-4 and IFN-γ Production by Lung Lymphocytes

The mediators (PGE_2, IL-5, MCP-1, and IL-12) could be produced by multiple cellular sources including eosinophils and parenchymal cells. To assess the activity of lymphocytes, we measured IL-4 and IFN-γ secretion ex vivo. Following BAL, lymphocytes were isolated from the lungs by enzyme digestion, and the cells were cultured with autologous sera. IL-4 expression was undetectable in cells cultured with medium alone, while in cells stimulated with PMA and ionomycin, IL-4 production was significantly higher in lymphocytes from the cholesterol group than from mice of the control diet group (493 ± 310 vs. 118 ± 119 pg/ml, $p = 0.04$) (fig. 1). Similarly, baseline IFN-γ production (258 ± 169 vs. 63 ± 91 pg/ml, $p = 0.03$) and PMA-stimulated IFN-γ production (1,895 ± 2,007 vs. 736 ± 1,070 pg/ml, $p = 0.07$) were both higher in lymphocytes isolated from the cholesterol group. Pravastatin did not significantly reduce the production of either of these two lymphokines. Again, lymphocyte production of IL-4 ($r = 0.64$, $p = 0.002$) and IFN-γ ($r = 0.60$, $p = 0.01$) were each correlated with serum cholesterol levels. In addition to lymphokine production, splenic lymphocyte proliferation (stimulated with phytohemagglutinin, PHA) was also 4.76 times as high in mice of the cholesterol group as in the control diet group (not shown in the figure).

LDL Supplement Increased PGE_2 and MCP-1

The data suggest that dietary cholesterol may lead to a pulmonary milieu that is more favorable for the development of Th2 cells, consisting of increased PGE_2 and MCP-1, with a concomitant reduction in IL-12. However, in this animal model it was not possible to discern if the altered PGE_2 and IL-12 expression was a direct result of cholesterol. We therefore tested the effect of cholesterol

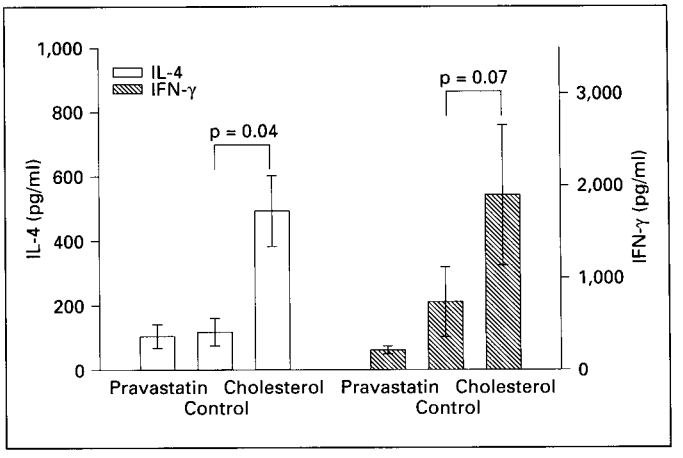

Fig. 1. Effect of dietary cholesterol on cytokine production by lung lymphocytes ex vivo. Lymphocytes (1.25×10^6 cells/well) from OVA-primed and challenged mice were isolated from enzyme-digested lung tissue by gradient isolation, and stimulated with 10 ng/ml PMA plus 1 μg/ml ionomycin for 24 h. IL-4 and IFN-γ production (mean ± SEM) was significantly higher in the cholesterol group ($n = 8$) than the control group ($n = 8$) by Wilcoxon rank sum test. There was no significant difference between cells from control diet group and the pravastatin group ($n = 5$).

(in the form of LDL) on the production of PGE_2 in vitro. RAW264.7 and A549 cells were incubated with 10, 25, 50, or 100 μg/ml LDL for 24 h, and spontaneous release of PGE_2 was measured. LDL dose-dependently increased PGE_2 production in both cell lines (fig. 2). Similarly, MCP-1 production by THP-1 cells increased with 50 μg/ml LDL in the medium, and the effect was more prominent when LDL was oxidized (fig. 2). We were unable to detect IL-12 (p70) in culture medium of mouse-macrophage cell lines RAW 264.7 and J774.

Discussion

In this study, we attempted to elucidate the mechanism of enhanced pulmonary allergic inflammation induced by dietary cholesterol. The results suggested that there might be more than one pathway. First, dietary cholesterol seemed to result in enhanced expression of inflammatory mediators, including IL-5, PGE_2, and MCP-1. Second, cholesterol altered the activities of lymphocytes, as reflected by IL-4 and IFN-γ production. Third, the production of IL-12 in the lungs was reduced. The combined effect of these changes was a microenvironment favorable for allergic inflammation.

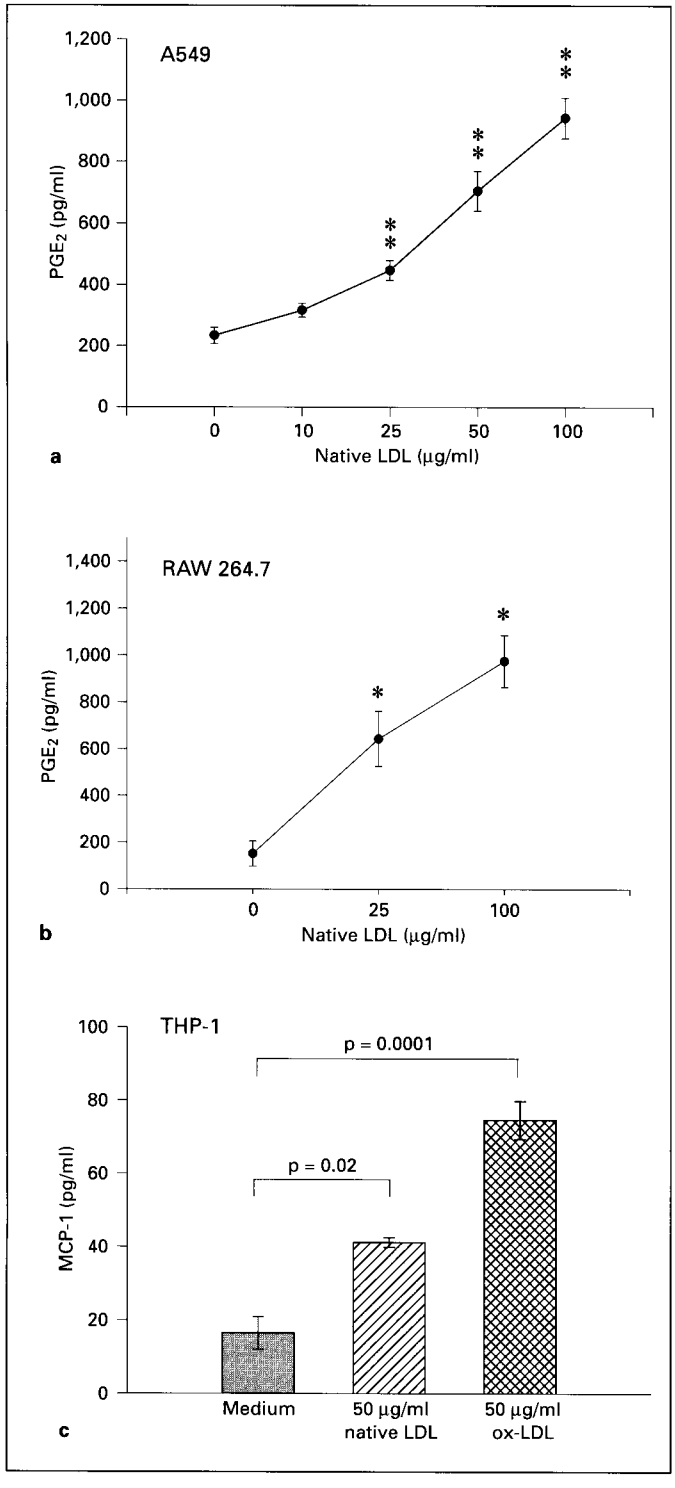

Fig. 2. Effect of LDL or oxidized LDL incubation on PGE_2 production by macrophages and epithelial cells and MCP-1 production by monocyte. A549 cells (**a**), RAW 264.7 (**b**), and THP-1 cells (**c**) 2.5×10^5 cells/well in 24-well culture plate were treated with medium alone, or with different concentrations of native or oxidized LDL (mean ± SEM). Significance according to Student's t test compared with the control medium: * $p < 0.05$; ** $p < 0.01$.

Dietary cholesterol caused a variety of changes in the inflammatory processes, but it is difficult in this animal model to determine which was the primary alteration leading to other downstream responses. In mice that were not challenged with OVA, cholesterol did not cause any significant alteration in the level of pulmonary inflammatory mediators. We did observe that, in these mice, splenic lymphocytes showed higher proliferation in response to PHA stimulation in vitro, indicating that lymphocyte functional change occurred prior to OVA inhalation. At this stage, however, IL-4 and IFN-γ release could not be demonstrated. After OVA inhalation, we observed increased IL-4 and IFN-γ production of lung lymphocytes ex vivo, suggesting that lymphocytes were functionally activated, both in Th1 and Th2 capacities. Here, the IFN-γ data seem to conflict with the reduced IL-12 levels detected in lung homogenate, because IL-12 is a potent inducer of IFN-γ. A possible explanation is that there is a priming effect of cholesterol on lymphocytes, but how the T cells respond remains to be determined by other factors, in this case a Th2 response predetermined by the conditions of antigen sensitization and challenge. The discrepancy could also result from the fact that lymphocytes stimulated in vitro may not adequately represent the complicated cytokine interactions in vivo. For instance, the effect of LDL on IL-12 and IFN-γ production by mononuclear cells could be affected by the presence of IL-10 [2].

We speculate that cholesterol might alter lymphocyte function through its effect on lipid rafts. Dietary cholesterol could increase the cholesterol contents of the plasma membrane [5], which plays an important role in maintaining lipid rafts. Some acylated proteins and glycosylphosphatidylinositol-linked proteins that participate in receptor-signaling pathways are either partitioned into rafts or become raft-associated when activated [1]. It remains to be determined, however, whether dietary cholesterol may actually affect the assembly and function of raft-associated proteins.

Although dietary cholesterol seemed to enhance inflammatory mediators (e.g., PGE_2, MCP-1, and IL-5), IL-12 was a significant exception. We were unable to discern whether cholesterol had a direct inhibitory effect on IL-12 production by in vitro experiments. Another possibility for how cholesterol might affect IL-12 is through PGE_2. PGE_2 secreted by epithelial cells and macrophages dose-dependently inhibited the ability of myeloid dendritic cells to produce IL-12 [8]. We found higher PGE_2 in the BALF of mice in the cholesterol group, and the direct effect of LDL to enhance PGE_2 production in both macrophage and epithelial cell lines; these findings were consis-

tent with a previous finding that cholesterol increased PGE_2 production in a human monocyte cell line [16]. Although it is not possible to know if altered PGE_2 production occurred prior to or following lymphocyte functional changes, our data suggested that PGE_2 could be a primary response to dietary cholesterol. Including a group of animals treated with cyclooxygenase inhibitors in future experiments will help to resolve the interaction among cholesterol, PGE_2, and IL-12.

We showed that MCP-1 was higher in the BALF of mice in the cholesterol group, and MCP-1 production by THP-1 monocytes in vitro were enhanced by treatment of LDL and oxidized LDL. Increased MCP-1 may contribute to the severity of pulmonary inflammation, because neutralization of MCP-1 before antigen challenge diminished airway hyperresponsiveness and inflammation in a murine model of asthma [3]. MCP-1 has also been implicated in T-cell deviation during antigen activation, leading to Th2 cytokine production [4, 10] and reduced production of IL-12 in response to CD40 ligand [18]. LDL treatment in vitro also induced a rapid increase in MCP-1 and CCR2 expression in human monocytes [5, 12, 17]. These changes may be relevant to the relationship between serum cholesterol and allergic responses in humans. Expression of MCP-1 and its receptor CCR2 by monocytes was dramatically increased in hypercholesterolemic patients compared with normocholesterolemic controls [5].

We showed in this mouse model that an HMG-CoA reductase inhibitor, pravastatin, could suppress allergic inflammation. It is not clear whether this suppression was due to its effect on lowering serum cholesterol or to its anti-inflammatory effect (such as inhibiting lymphocyte proliferation, reducing MHC expression, or inhibiting integrin-mediated cell adhesion [13, 20, 22]). Although the inhibitory effect of pravastatin is most likely due to a combination of these two mechanisms, the data showed clearly that all indicators of inflammation were correlated with serum cholesterol levels, suggesting the importance of the cholesterol-lowering effect. In a murine model of collagen-induced arthritis, which is Th1-driven, simvastatin suppressed the inflammatory process and inhibited the development and clinical manifestation of arthritis [14]. To our knowledge, our study is the first to explore the effect of a statin on a Th2-driven disease model.

It is to be noted that not all rodent species and strains become hyperlipidemic in response to dietary cholesterol supplement. With our protocol, 2% dietary cholesterol raised serum cholesterol levels by 36%, but the mice were not overtly hyperlipidemic. On the other hand, pravastatin decreased serum cholesterol levels by approximately 25%. Our studies in mice were conducted at a dose (16–20 mg/kg) higher than that used in humans (1–2 mg/kg); however, the serum concentration may be similar to therapeutic range in humans, due to higher pravastatin metabolism rate and a rapid up-regulation of HMG-CoA reductase in rodents [11].

In summary, dietary cholesterol enhanced pulmonary allergic inflammation, with elevated expression of PGE_2, MCP-1, and IL-5 that participate in the recruitment and activation of monocytes and eosinophils. Dietary cholesterol may contribute to the enhancement of lymphocyte activity or a microenvironment favoring Th2 inflammation. However, this latter effect must be viewed in the context of antigen sensitization and challenge, in that cholesterol may enhance preexisting inflammatory processes, whether it is Th1- or Th2-driven. In addition, pravastatin was shown to effectively inhibit pulmonary allergic inflammation, either by its effect on lowering serum cholesterol or by its direct immunomodulating effect. These results suggest the significance of dietary modulation and the potential therapeutic effect of statins on asthmatic patients.

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References

1 Dykstra M, Cherukuri A, Sohn HW, Tzeng SJ, Pierce SK. Location is everything: Lipid rafts and immune cell signaling. Annu Rev Immunol 21:457–481;2003.

2 Fei G-Z, Huang Y-H, Swedenborg J, Frostegard J. Oxidised LDL modulates immune-activation by an IL-12 dependent mechanism. Atherosclerosis 169:77–85;2003.

3 Gonzalo JA, Lloyd CM, Wen D, Albar JP, Wells TN, Proudfoot A, Martinez AC, Dorf M, Bjerke T, Coyle AJ, Gutierrez-Ramos JC. The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. J Exp Med 188:157–167;1998.

4 Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. Nature 404:407–411;2000.

5 Han KH, Tangirala RK, Green SR, Quehenberger O. Chemokine receptor CCR2 expression and monocyte chemoattractant protein-1-mediated chemotaxis in human monocytes. A regulatory role for plasma LDL. Arterioscler Throm Vasc Biol 18:1983–1991;1998.

6 Huang S-L, Lin K-C, Pan W-H. Dietary factors associated with physician-diagnosed asthma and allergic rhinitis in teenagers – Analysis of the first Nutrition and Health Surveillance in Taiwan. Clin Exp Allergy 301:259–264;2001.

7 Huang S-L, Shiao G-M, Chou P. Association between body mass index and allergy in teenage girls in Taiwan. Clin Exp Allergy 28:323–329;1999.

8 Kalinski P, Hilkens CM, Snijders A, Snijdewint FG, Kapsenberg ML. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. J Immunol 159:28–35;1997.

9 Kalinski P, Schuitemaker JH, Hilkens CM, Kapsenberg ML. Prostaglandin E2 induces the final maturation of IL-12-deficient CD1a+CD83+ dendritic cells: The levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. J Immunol 161:2804–2809;1998.

10 Karpus WJ, Lukacs NW, Kennedy KJ, Smith WS, Hurst SD, Barrett TA. Differential CC chemokine-induced enhancement of T helper cell cytokine production. J Immunol 158: 4129–4136;1997.

11 Kita T, Brown MS, Goldstein JL. Feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in livers of mice treated with mevinolin, a competitive inhibitor of the reductase. J Clin Invest 66:1094–1100;1980.

12 Klouche M, Gottschling S, Gerl V, Hell W, Husmann M, Dorweiler B, Messner M, Bhakdi S. Atherogenic properties of enzymatically degraded LDL: Selective induction of MCP-1 and cytotoxic effects on human macrophages. Arterioscler Throm Vasc Biol 18:1376–1385; 1998.

13 Kwak B, Mulhaupt F, Myit S, Mach F. Statins as a newly recognized type of immunomodulator. Nat Med 6:1399–1402;2000.

14 Leung BP, Sattar N, Crilly A, Prach M, McCarey DW, Payne H, Madhok R, Campbell C, Gracie JA, Liew FY, McInnes IB. A novel anti-inflammatory role for simvastatin in inflammatory arthritis. J Immunol 170:1524–1530; 2003.

15 Luther SA, Cyster JG. Chemokines as regulators of T cell differentiation. Nat Immunol 2: 102–107;2001.

16 Morris RH, Price AJ, Tonks A, Jackson SK, Jones KP. Prostaglandin E(2) and tumour necrosis factor-alpha release by monocytes are modulated by phospholipids. Cytokine 12: 1717–1719;2000.

17 Okada M, Suzuki A, Mizuno K, Asada Y, Ino Y, Kuwayama T, Tamakoshi K, Mizutani S, Tomoda Y. Effects of 17 beta-estradiol and progesterone on migration of human monocytic THP-1 cells stimulated by minimally oxidized low-density lipoprotein in vitro. Cardiovasc Res 34:529–535;1997.

18 Omata N, Yasutomi M, Yamada A, Iwasaki H, Mayumi M, Ohshima Y. Monocyte chemoattractant protein-1 selectively inhibits the acquisition of CD40 ligand-dependent IL-12-producing capacity of monocyte-derived dendritic cells and modulates Th1 immune response. J Immunol 169:4861–4866;2002.

19 Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu YJ. Reciprocal control of T helper cell and dendritic cell differentiation. Science 283:1183–1186;1999.

20 Rudich SM, Mongini PK, Perez RV, Katznelson S. HMG-CoA reductase inhibitors pravastatin and simvastatin inhibit human B-lymphocyte activation. Transplant Proc 30:992–995;1998.

21 Smits HH, de Jong EC, Schuitemaker JH, Geijtenbeek TB, van Kooyk Y, Kapsenberg ML, Wierenga EA. Intercellular adhesion molecule-1/LFA-1 ligation favors human Th1 development. J Immunol 168:1710–1716;2002.

22 Weitz-Schmidt G, Welzenbach K, Brinkmann V, Kamata T, Kallen J, Bruns C, Cottens S, Takada Y, Hommel U. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. Nat Med 7: 687–692;2001.

23 Yeh Y-F, Huang S-L. Dietary cholesterol enhances pulmonary eosinophilic inflammation in a murine model of asthma. Int Arch Allergy Immunol 125:329–334;2001.