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The effect of ezetimibe, administered alone or in combination with simvastatin, on lymphocyte cytokine release in patients with elevated cholesterol levels

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Abstract. Krysiak R, Zmuda W, Okopien B (Medical University of Silesia, Katowice; and and Electrotherapy and Angiology Centre, Oswiecim, Poland). The effect of ezetimibe, administered alone or in combination with simvastatin, on lymphocyte cytokine release in patients with elevated cholesterol levels. *J Intern Med* 2012; **271**: 32–42.

Objective. Studies assessing the extra-lipid effects of ezetimibe have provided contrasting results. In the present study, we compared the effects of ezetimibe and simvastatin, administered alone or in combination, on the secretory function of human lymphocytes, systemic inflammation and endothelial function in subjects with elevated cholesterol levels.

Methods. A prospective study involving a group of 178 ambulatory patients with isolated hypercholesterolaemia who were randomly assigned in a double-blind fashion to 90 days of treatment with ezetimibe (10 mg), simvastatin (40 mg), ezetimibe (10 mg) plus simvastatin (40 mg) or placebo. A total of 170 patients completed the study.

Main outcome measures. Lymphocyte cytokine release and plasma levels of high-sensitivity C-reactive protein (hsCRP) and intercellular adhesion molecule 1 (ICAM-1).

Results. Although both drugs reduced lymphocyte release of tumour necrosis factor-α, interferon-γ and interleukin-2 in a lipid-independent manner, only the effect of simvastatin was statistically significant ($P < 0.001$). This lymphocyte-suppressing effect, which was accompanied by a decrease in plasma levels of hsCRP and ICAM-1 ($P < 0.001$), was strongest in patients receiving both simvastatin and ezetimibe. There were no differences in lymphocyte-suppressing, systemic anti-inflammatory and endothelial protective effects of simvastatin between insulin-resistant and insulin-sensitive subjects, whereas the effects of ezetimibe and the combined treatment were greater in the former group of patients ($P < 0.01$ and $P < 0.001$, respectively).

Conclusions. The results of this study indicate that simvastatin is superior to ezetimibe in producing lymphocyte-suppressing, systemic anti-inflammatory and endothelial protective effects in patients with elevated cholesterol levels. Hypercholesterolaemic patients with high cardiovascular risk may receive the greatest benefits from concomitant treatment with a statin and ezetimibe.

Keywords: cytokines, ezetimibe, hypercholesterolaemia, lymphocytes, simvastatin, systemic inflammation.

Abbreviations: CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA, homeostasis model assessment index; hsCRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule-1; LDL, low-density lipoprotein; NPC1L1, Niemann–Pick C1-like 1; TNF-α, tumour necrosis factor-α.

Introduction

Inflammation, involving all the cellular elements of the vascular wall, i.e. endothelial cells, smooth muscle cells and immune cells, is generally considered to be one of the major factors responsible for the development and progression of atherosclerosis [1–3]. Those inflammatory processes play a crucial role in atherogenesis, which is reflected by the presence of large numbers of inflammatory cells, mainly monocytes/macrophages and T lymphocytes, within the atherosclerotic plaque [2, 3]. The dominant subset of T cells found in the plaque, namely CD4+ helper cells,

recognizes antigens associated with class II major histocompatibility complex molecules, and the pattern of local cytokine secretion suggests a T helper type 1 response [4]. Therefore, treatment with hypolipidaemic agents exerting additional anti-inflammatory effects may offer additional benefits to patients with atherosclerosis compared with drugs affecting only the lipid/lipoprotein profile.

The novel hypolipidaemic agent ezetimibe, which inhibits Niemann–Pick C1-like 1 (NPC1L1) transport protein (a critical protein in cholesterol transmembrane transport in the small intestine) in the brush border of enterocytes, is a strong cholesterol and phytosterol absorption inhibitor [5, 6]; however, its actions may not be limited to improving plasma lipids. Unfortunately, conflicting results have been provided by the few studies that have investigated the anti-inflammatory effects of this agent. In some studies, ezetimibe produced a multidirectional anti-inflammatory effect [7–9], whereas in others this effect was either weak [10] or absent [11]. Recent studies have provided some evidence that ezetimibe may affect the number and function of the cells directly involved in the process of atherogenesis, mainly macrophages and lymphocytes. Monocyte-derived macrophages have been found to express target proteins for ezetimibe: NPC1L1, aminopeptidase N, annexin-2 and caveolin-1 [12, 13]. Ezetimibe was found to reduce monocyte expression of raft-associated antigens and to induce transfer of aminopeptidase N from plasma membrane to intracellular vesicles [13]. This mechanism may be responsible for a decrease in lipid accumulation in the atheromatous plaque. Gómez-Garre *et al.* [14] observed that ezetimibe, either alone or in combination with simvastatin, reduced the number of monocytes/macrophages in atherosclerotic lesions (particularly in patients receiving both these agents), reduced monocyte chemoattractant protein 1 expression in atherosclerotic lesions and inhibited the migratory response of monocytes in a rabbit model of atherosclerosis. To the best of our knowledge, only one study has assessed the effect of this agent on T cells, showing that ezetimibe exerts immunomodulatory properties. When administered to cardiac transplant recipients, the drug reduced the number of CD3+ CD4+ T cells and CD3+ CD4+ CD45RO T memory cells in a lipid-independent manner, and this effect was similar to that produced by atorvastatin [15]. Because of the paucity of data and the fact that *in vitro* conditions cannot easily be translated to conditions in human patients, we conducted this prospective, randomized, placebo-controlled study to investigate whether ezetimibe, administered alone or in combination with simvastatin, affects lymphocyte cytokine release and whether this effect is involved in the systemic anti-inflammatory and endothelial protective effect of this agent.

Subjects and methods

Subjects

Study participants were recruited amongst individuals screened in our department as a reference unit for the presence of an abnormal lipid/lipoprotein profile. Patients (20–70 years old) were eligible for the study if they had recently been diagnosed with and were previously untreated for isolated hypercholesterolaemia, defined as levels of plasma total cholesterol >200 mg dL^{-1}, low-density lipoprotein (LDL) cholesterol >130 mg dL^{-1} and triglycerides <150 mg dL^{-1}. The exclusion criteria are presented in the Data S1.

Study design

The study was reviewed and approved by the institutional ethics committee and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent to participate. All patients who met the eligibility criteria received counselling regarding how to follow the therapeutic lifestyle changes diet and were invited after 6 weeks of following this diet to repeat the lipid profile evaluation. These subjects, in whom the second test confirmed the results of the first one (n = 178), were then randomly assigned in a double-blind fashion to receive ezetimibe (10 mg daily; n = 45), simvastatin (40 mg daily; n = 46), ezetimibe (10 mg daily) plus simvastatin (40 mg daily; n = 45) or placebo (n = 42). A computer program was used for randomization. Each treatment group was divided into two subgroups: patients with and without normal insulin sensitivity. Normal insulin sensitivity was arbitrarily defined as the homeostasis model assessment (HOMA) index <2.0. If the HOMA index exceeded this threshold value, the patient was considered to be insulin resistant. Patients and all study personnel were blinded to treatment assignment. Both ezetimibe and simvastatin were administered once daily for 12 weeks without any changes in dosage during the entire study period. To minimize the risk of eventual pharmacokinetic interactions between ezetimibe and simvastatin, both drugs were administered at 12-h intervals. If patients were already taking other drugs, their pharmacological schedule remained constant throughout

the study. During the entire study period, all included patients continued to follow the therapeutic lifestyle changes diet. The possibility of ezetimibe- and/or simvastatin-induced side effects was assessed fortnightly. Compliance was assessed during each visit by tablet counts and was considered satisfactory when the number of tablets taken by a patient ranged from 90% to 110% of the total.

The primary study objective was to evaluate lymphocyte-suppressing, systemic anti-inflammatory and endothelial protective effects of ezetimibe using a panel of inflammation markers: tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-2 (IL-2), high-sensitivity C-reactive protein (hsCRP) and intercellular adhesion molecule 1 (ICAM-1). In addition, the effect of combination therapy of ezetimibe and simvastatin on these inflammation markers was determined.

Laboratory assays

Venous blood was collected at baseline (after 6 weeks of lifestyle modification), after 4 weeks of therapy and at the end of the treatment period. Samples were taken 12 h after a meal, always between the hours of 8.00 and 9.00, to avoid circadian fluctuations of the studied parameters and immediately coded so that the person performing the laboratory assay was blinded to subject identity and study sequence. To minimize analytical errors, all assays were carried out in duplicate. Routine chemical methods were used to determine plasma concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, triglycerides and glucose (colorimetric enzymatic method; bioMerieux, Marcy-l'Etoile, France; Beckman, Palo Alto, CA, USA). To avoid any error resulting from the Friedewald formula, LDL cholesterol was determined directly. Apoprotein A-I and apoprotein B levels were assessed by immunoturbidimetry (Incstar Corp., Stillwater, MN, USA). Plasma insulin concentration was measured with a commercial radioimmunoassay kit with no cross-reactivity with human proinsulin (Linco Research Inc, St Charles, MO, USA). To estimate insulin resistance, the HOMA index was calculated using the following equation: [fasting serum glucose (mg dL^{-1}) $\times$ fasting insulin level (μU mL^{-1})]/405. Plasma C-reactive protein levels were assessed by a highly sensitive immunoassay using monoclonal antibodies obtained from MP Biomedicals (Orangeburg, NY, USA). Phytohaemagglutinin-stimulated T cells were cultured in triplicate as previously described [16]. TNF-α, IFN-γ, IL-2 release and plasma soluble ICAM-1 levels were measured with commercial enzyme-linked immunosorbent assay kits obtained from R&D Systems (McKinley Place N.E. Minneapolis, MN, USA). The minimum detectable levels for hsCRP, TNF-α, IFN-γ, IL-2 and ICAM-1 were 0.1 ng mL^{-1}, 4.4, 15, 8 pg mL^{-1} and 0.096 ng mL^{-1}, respectively. The intra- and interassay coefficients of variation for all the assessed markers were below 4.8% and 8.7%, respectively.

Power calculations

A power analysis was conducted prior to the study using the Sample Power software (SPSS, Chicago, IL, USA) on the basis of the data from our previous study [16] and from an earlier pilot study conducted by our team (data not shown). Assuming a power of 80% and a significance (α) level of 0.05, at least 32 subjects would need to be randomly assigned to each treatment group (at least 16 subjects to each subgroup) to detect a 20% difference between the groups in all aspects of the primary end-point. Assuming possible dropouts as well as estimation and measurement inaccuracies, the sample size was increased to more than 40 patients per group.

Statistical analyses

All statistical analyses were performed using GraphPad Prism 2.01 (GraphPad, Software Inc., San Diego, CA, USA) and Statistica 6.1 software (StatSoft, Tulsa, OK, USA). Statistical significance was assumed at $P < 0.05$. First, the Kolmogorov–Smirnov test was used to analyse the normality of the distribution of the parameters measured. Results for the HOMA index and levels of TNF-α, IFN-γ, IL-2, hsCRP and ICAM-1 were natural-log transformed to satisfy assumptions of normality and equal variance. Because lipid, lipoprotein, carbohydrate and, after logarithmic transformation, all other values were normally distributed, parametric tests were used for statistical analysis. Treatment groups were compared using one-way anova followed by the *post hoc* Bonferroni test. The differences between baseline, inter- and post-treatment values within the same treatment group were compared with Student's paired *t*-test. Moreover, to verify the correctness of the statistical analysis, the median values of the HOMA index and levels of TNF-α, IFN-γ, IL-2, hsCRP and ICAM-1 were recalculated using nonparametric statistics (the Kruskal–Wallis test followed by the Mann–Whitney *U*-test and the Wilcoxon matched paired test), and the same results were obtained. For categorical variables, the chi-square test was applied.

Kendall's tau test was used to evaluate the relationship between metabolic variables and inflammatory mediators.

Results

Patient baseline characteristics

There were no differences in baseline characteristics between the treatment groups. Mean values of plasma lipids/lipoproteins, glucose homeostasis markers, lymphocyte cytokine release and plasma hsCRP and ICAM-1 levels were all comparable between the study groups (Table 1).

Adverse effects

Three participants, two allocated to simvastatin alone and one treated with simvastatin plus ezetimibe, experienced mild myalgia or other skeletal muscle problems and therefore discontinued treatment. None of these patients had values of creatine kinase that exceeded 10 times the upper limit of normal. Two individuals, one assigned to placebo and one receiving both ezetimibe and simvastatin, dropped out of the study because of noncompliance with the study protocol. One subject receiving ezetimibe also dropped out because of a treatment-associated increase in aminotransferases to more than

Table 1 *Baseline characteristics of patients*[a]

	Placebo	Ezetimibe	Simvastatin	Ezetimibe + simvastatin
Number of patients	41	43	44	42
Age (years; mean ± SD)	51 ± 3	50 ± 3	51 ± 4	52 ± 4
Women (%)	44	40	45	43
Body mass index (kg m^{-2}; mean ± SD)	27.8 ± 2.6	27.9 ± 2.8	28.2 ± 3.2	27.7 ± 2.5
Smokers (%)	32	33	34	36
Mild hypertension (%)	15	14	16	12
Insulin-resistant subjects (%)	46	51	48	52
Medications (%)				
β_1-adrenergic blockers	12	9	9	10
Imidazoline receptor agonists	2	5	7	2
Total cholesterol (mg dL^{-1}; mean ± SD)	252 ± 12	258 ± 14	259 ± 13	255 ± 12
Low-density lipoprotein cholesterol (mg dL^{-1}; mean ± SD)	179 ± 9	181 ± 9	183 ± 10	182 ± 8
High-density lipoprotein cholesterol (mg dL^{-1}; mean ± SD)	45 ± 4	46 ± 4	44 ± 3	45 ± 3
Triglycerides (mg dL^{-1}; mean ± SD)	119 ± 11	122 ± 11	120 ± 12	124 ± 12
Apoprotein A-I (mg dL^{-1}; mean ± SD)	123 ± 8	125 ± 5	126 ± 8	123 ± 7
Apoprotein B (mg dL^{-1}; mean ± SD)	172 ± 10	174 ± 8	175 ± 7	177 ± 8
Fasting glucose (mg dL^{-1}; mean ± SD)	96 ± 5	94 ± 5	93 ± 3	95 ± 5
2-h postglucose load plasma glucose (mg dL^{-1}; mean ± SD)	133 ± 7	135 ± 5	137 ± 6	138 ± 7
Homeostasis model assessment index (mean ± SD)	2.9 ± 0.4	2.9 ± 0.4	2.8 ± 0.5	3.0 ± 0.4
High-sensitivity C-reactive protein (mg L^{-1}; mean ± SD)	3.2 ± 0.4	3.4 ± 0.4	3.3 ± 0.4	3.5 ± 0.4
Intercellular adhesion molecule 1 (ng mL^{-1}; mean ± SD)	302 ± 32	305 ± 46	307 ± 31	299 ± 35
TNF-α release (pg mL^{-1}; mean ± SD)	348 ± 31	361 ± 26	364 ± 34	359 ± 38
IFN-γ release (ng mL^{-1}; mean ± SD)	53.2 ± 6.0	54.2 ± 7.1	54.4 ± 5.2	52.9 ± 6.4
IL-2 release (ng mL^{-1}; mean ± SD)	5.5 ± 0.6	5.6 ± 0.6	5.8 ± 0.5	5.7 ± 0.5

[a]Only data from subjects who completed the study were included in the final analyses.

three times the upper limit of normal. Another patient complained of abdominal pains and diarrhoea whilst on combination therapy and withdrew from the study. Additionally, a patient treated with ezetimibe refused to further participate in the study because of personal reasons. Neither significant adverse effects nor any other complications were reported throughout the study for the remaining 170 patients who completed the study protocol.

Lipid/lipoprotein profile and glucose homeostasis

Twelve weeks of placebo treatment did not affect either the lipid/lipoprotein profile or glucose homeostasis markers. Ezetimibe, simvastatin and the combination treatment decreased circulating levels of total cholesterol, LDL cholesterol and apoprotein B. When administered together, ezetimibe and simvastatin also increased plasma levels of HDL cholesterol and apoprotein A-I. None of the treatment options affected glucose homeostasis markers, although ezetimibe or ezetimibe plus simvastatin showed a tendency to reduce the HOMA index (ezetimibe: $P = 0.085$ and $P = 0.076$; ezetimibe plus simvastatin: $P = 0.087$ and $P = 0.075$ after 4 and 12 weeks of treatment, respectively) (Table 2).

Plasma hsCRP and ICAM-1

No changes in plasma hsCRP and ICAM-1 levels were observed during the entire study period in placebo-treated patients. Ezetimibe alone tended to reduce plasma levels of these proteins (hsCRP: $P = 0.076$ and $P = 0.053$; ICAM-1: $P = 0.069$ and $P = 0.059$ after 4 and 12 weeks of treatment, respectively). Both 4 and 12 weeks of simvastatin therapy alone or in combination with ezetimibe decreased plasma hsCRP and ICAM-1 levels. Simvastatin- and combination therapy-induced changes in plasma hsCRP and ICAM-1 were more pronounced at the end of the study than after 4 weeks of treatment (Table 3).

Lymphocyte cytokine release

Placebo treatment was without any effect on lymphocyte cytokine release. Ezetimibe treatment was associated with a trend towards a decrease in phytohaemagglutinin-induced cytokine release (TNF-α: $P = 0.089$ and $P = 0.078$; IFN-γ: $P = 0.084$ and $P = 0.059$; IL-2: $P = 0.087$ and $P = 0.051$ after 4 and 12 weeks of treatment, respectively). Four and 12 weeks of treatment of hypercholesterolaemic patients with simvastatin alone or in combination with ezetimibe reduced lymphocyte release of all cytokines studied. The effect of simvastatin alone or the combination therapy on cytokine release was stronger after 12 than after 4 weeks of treatment (Table 3).

Subgroup analysis

Insulin-sensitive patients. Simvastatin, administered alone or in combination with ezetimibe, to patients with a normal HOMA index reduced cytokine release and circulating levels of hsCRP and ICAM-1, whereas no effect was observed with ezetimibe alone (Table 4).

Insulin-resistant patients. In insulin-resistant patients, all treatment options reduced cytokine release and plasma levels of the assessed variables.

Between-group comparisons

The combination therapy was superior to the other treatment options with regard to circulating levels of total cholesterol, LDL cholesterol, apoprotein B, hsCRP and ICAM-1, as well as lymphocyte cytokine release. Simvastatin or ezetimibe administered alone was superior to placebo in reducing total and LDL cholesterol and apoprotein B levels and, for simvastatin, in reducing plasma hsCRP and ICAM-1 levels as well as cytokine release. Simvastatin alone was superior to ezetimibe alone in reducing plasma hsCRP and ICAM-1 levels and lymphocyte cytokine release. The effects of ezetimibe alone or the combination therapy, but not of simvastatin alone, on cytokine release and circulating levels of hsCRP and ICAM-1 were more evident in insulin-resistant than in insulin-sensitive patients (Tables 3 and 4).

Correlations

At entry, plasma hsCRP levels showed a weak correlation with lymphocyte release of TNF-α ($r = 0.49$, $P < 0.001$), IFN-γ ($r = 0.56$, $P < 0.001$) and IL-2 ($r = 0.50$, $P < 0.001$), as well as with plasma soluble ICAM-1 ($r = 0.52$, $P < 0.001$). There was no correlation between lipid/lipoprotein profile and cytokine release or plasma levels of hsCRP and ICAM-1. Plasma ICAM-1 level did not correlate with cytokine release. The effect of simvastatin, ezetimibe and the combination therapy on hsCRP correlated weakly with their effect on cytokine release (simvastatin: $r = 0.47$–0.59, $P < 0.001$; ezetimibe: $r = 0.46$–0.57, $P < 0.001$; ezetimibe plus simvastatin: $r = 0.53$–0.62, $P < 0.001$) and on plasma ICAM-1 (simvastatin: $r = 0.60$, $P < 0.001$; ezetimibe: $r = 0.53$, $P < 0.001$; ezetimibe plus simvastatin: $r = 0.61$, $P < 0.001$).

Table 2 *The effect of ezetimibe and simvastatin on lipid/lipoprotein profile and glucose homeostasis in patients with isolated hypercholesterolaemia**

	Placebo	Ezetimibe	Simvastatin	Ezetimibe + simvastatin
Total cholesterol (mg dL^{-1})				
Baseline	252 ± 12	258 ± 14	259 ± 13	255 ± 12
After 4 weeks	255 ± 13 (1)	199 ± 12 (–23)[b,d]	194 ± 12 (–25)[b,d]	159 ± 9 (–38)[b,d,e,g]
After 12 weeks	258 ± 15 (2)	198 ± 13 (–23)[b,d]	191 ± 19 (–26)[b,d]	157 ± 11 (–39)[b,d,f,g]
Low-density lipoprotein cholesterol (mg dL^{-1})				
Baseline	179 ± 9	181 ± 9	183 ± 10	182 ± 8
After 4 weeks	181 ± 8 (1)	137 ± 8 (–24)[b,d]	125 ± 8 (–32)[b,d]	99 ± 8 (–46)[b,d,f,g]
After 12 weeks	176 ± 12 (–2)	134 ± 7 (–26)[b,d]	122 ± 9 (–33)[b,d]	95 ± 6 (–48)[b,d,f,g]
High-density lipoprotein cholesterol (mg dL^{-1})				
Baseline	45 ± 4	46 ± 4	44 ± 3	45 ± 3
After 4 weeks	43 ± 4 (–4)	44 ± 4 (–4)	48 ± 5 (9)	54 ± 3 (20)[a,c]
After 12 weeks	45 ± 4 (0)	47 ± 5 (2)	47 ± 4 (7)	54 ± 3 (20)[a,c]
Triglycerides (mg dL^{-1})				
Baseline	119 ± 11	122 ± 11	120 ± 12	124 ± 12
After 4 weeks	125 ± 14 (5)	115 ± 12 (–6)	111 ± 13 (–8)	110 ± 10 (–11)
After 12 weeks	127 ± 13 (7)	112 ± 10 (–8)	111 ± 14 (–8)	109 ± 11 (–12)
Apoprotein A-I (mg dL^{-1})				
Baseline	123 ± 8	125 ± 5	126 ± 8	123 ± 7
After 4 weeks	121 ± 7 (–2)	123 ± 8 (–2)	131 ± 9 (4)	142 ± 5 (15)[a,c]
After 12 weeks	124 ± 7 (1)	129 ± 8 (3)	131 ± 6 (4)	143 ± 6 (16)[a,c]
Apoprotein B (mg dL^{-1})				
Baseline	172 ± 10	174 ± 8	175 ± 7	177 ± 8
After 4 weeks	175 ± 13 (2)	143 ± 7 (–18)[a,c]	132 ± 6 (–25)[b,d]	114 ± 5 (–36)[b,d,f,g]
After 12 weeks	176 ± 12 (2)	141 ± 7 (–19)[a,c]	130 ± 7 (–26)[b,d]	111 ± 5 (–37)[b,d,f,g]
Fasting glucose (mg dL^{-1})				
Baseline	96 ± 5	94 ± 5	93 ± 3	95 ± 5
After 4 weeks	98 ± 5 (2)	93 ± 3 (–1)	95 ± 4 (2)	93 ± 4 (–2)
After 12 weeks	97 ± 4 (1)	92 ± 4 (–2)	95 ± 4 (2)	93 ± 3 (–2)
2-h postglucose load plasma glucose (mg dL^{-1})				
Baseline	133 ± 7	135 ± 5	137 ± 6	138 ± 7
After 4 weeks	135 ± 6 (2)	132 ± 5 (–2)	139 ± 8 (1)	136 ± 7 (–1)
After 12 weeks	136 ± 7 (2)	130 ± 6 (–4)	138 ± 6 (1)	133 ± 6 (–4)
Homeostasis model assessment index				
Baseline	2.9 ± 0.4	2. 9 ± 0.4	2.8 ± 0.5	3.0 ± 0.4
After 4 weeks	3.0 ± 0.4 (3)	2.5 ± 0.5 (–14)	3.0 ± 0.5 (7)	2.6 ± 0.5 (–13)
After 12 weeks	2.9 ± 0.4 (0)	2.4 ± 0.4 (–17)	2.9 ± 0.4 (4)	2.5 ± 0.4 (–17)

Data represent the mean ± SD. Values in parentheses represent percentage changes from baseline values. [a]$P < 0.05$, [b]$P < 0.001$ vs. control group. [c]$P < 0.05$, [d]$P < 0.001$ vs. pretreatment values. [e]$P < <0.05$, [f]$P < <0.01$ vs. ezetimibe-treated patients. [g]$P < 0.05$ vs. simvastatin-treated patients.
*Only data from subjects who completed the study were included in the final analyses.

Table 3 *The effect of ezetimibe and simvastatin on systemic inflammation, endothelial function and lymphocyte cytokine release in patients with isolated hypercholesterolaemia**

	Placebo	Ezetimibe	Simvastatin	Ezetimibe + simvastatin
High-sensitivity C-reactive protein (mg L^{-1})				
Baseline	3.2 ± 0.4	3.4 ± 0.4	3.3 ± 0.4	3.5 ± 0.4
After 4 weeks	3.3 ± 0.3 (3)	2.8 ± 0.3 (−18)	2.5 ± 0.2 (−22)[a,d]	2.0 ± 0.4 (−43)[c,f]
After 12 weeks	3.4 ± 0.5 (6)	2.7 ± 0.4 (−19)	1.9 ± 0.2 (−42)[c,f,h,l]	1.1 ± 0.2 (−69)[c,f,i,k,n]
Intercellular adhesion molecule 1 (ng mL^{-1})				
Baseline	302 ± 32	305 ± 46	307 ± 31	299 ± 35
After 4 weeks	306 ± 24 (1)	253 ± 31 (−17)	236 ± 18 (−23)[a,d]	202 ± 24 (−32)[c,f]
After 12 weeks	308 ± 26 (2)	247 ± 23 (−19)	196 ± 15 (−36)[c,f,g,l]	147 ± 16 (−51)[c,f,i,j,m]
TNF-α release (pg mL^{-1})				
Baseline	348 ± 31	361 ± 26	364 ± 34	359 ± 38
After 4 weeks	361 ± 33 (4)	305 ± 28 (−16)	280 ± 25 (−23)[a,d]	241 ± 22 (−33)[c,f]
After 12 weeks	365 ± 31 (5)	298 ± 32 (−17)	220 ± 21 (−40)[c,f,g,l]	175 ± 23 (−51)[c,f,i,j,m]
IFN-γ release (ng mL^{-1})				
Baseline	53.2 ± 6.0	54.2 ± 7.1	54.4 ± 5.2	52.9 ± 6.4
After 4 weeks	52.8 ± 7.4 (−1)	45.6 ± 3.7 (−16)	41.6 ± 3.5 (−24)[a,e]	36.7 ± 5.1 (−31)[b,f]
After 12 weeks	52.4 ± 4.4 (−2)	44.5 ± 4.5 (−18)	32.7 ± 3.2 (−40)[c,f,h,l]	24.8 ± 2.2 (−53)[c,f,i,j,n]
IL-2 release (ng mL^{-1})				
Baseline	5.5 ± 0.6	5.6 ± 0.6	5.8 ± 0.5	5.7 ± 0.5
After 4 weeks	5.5 ± 0.5 (0)	4.6 ± 0.5 (−18)	4.5 ± 0.3 (−22)[a,d]	3.9 ± 0.4 (−32)[c,f]
After 12 weeks	5.7 ± 0.5 (4)	4.5 ± 0.5 (−19)	3.7 ± 0.2 (−36)[c,f,g,l]	2.9 ± 0.3 (−49)[c,f,i,j,m]

Data represent the mean ± SD. Values in parentheses represent percentage changes from baseline values. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ vs. control group. [d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$ vs. pretreatment values. [g]$P < 0.05$, [h]$P < 0.01$, [i]$P < 0.001$ vs. ezetimibe-treated patients. [j]$P < 0.05$, [k]$P < 0.001$ vs. simvastatin-treated patients. [l]$P < 0.05$, [m]$P < 0.01$, [n]$P < 0.001$ vs. the effect after 4 weeks of treatment.
*Only data from subjects who completed the study were included in the final analyses.

There was a correlation between ezetimibe- and the combination therapy-induced changes in the HOMA index and the effect on cytokine release and plasma levels of hsCRP and ICAM-1 (ezetimibe: $r = 0.48$–0.59, $P < 0.001$; ezetimibe plus simvastatin: $r = 0.46$–0.58, $P < 0.001$). The treatment-induced reductions in levels of hsCRP and ICAM-1 and lymphocyte cytokine release were unrelated to the degree of lipid/lipoprotein profile improvement and, for simvastatin, to the action on glucose homeostasis markers. The treatment-induced reduction in ICAM-1 did not correlate with the effect of either drug on cytokine release.

Discussion

The major finding of our study is that simvastatin is superior to ezetimibe in producing lymphocyte-suppressing, systemic anti-inflammatory and endothelial protective effects in patients with hypercholesterolaemia. The strongest effect was observed when both these agents were administered together, which suggests that combined treatment with ezetimibe and simvastatin is an interesting therapeutic option in high-risk patients with hypercholesterolaemia.

In line with our previous studies [16, 17], simvastatin significantly reduced lymphocyte cytokine release, with the strength of the effect determined by the length of treatment. Because simvastatin was administered in the same dose as used in the Heart Protection Study [18], the largest study that demonstrated the benefits of statin use in the prevention of cardiovascular disease, a lymphocyte-suppressing effect of this agent may in part explain why statins delay the development and progression of atherosclerosis. In turn, ezetimibe showed only a tendency to affect lymphocyte cytokine release. Although both statins [19]

Table 4 *The effect of ezetimibe and simvastatin on plasma hsCRP and ICAM-1 levels, and on lymphocyte cytokine release in insulin-sensitive and insulin-resistant subjects with isolated hypercholesterolaemia**

	Ezetimibe		Simvastatin		Ezetimibe + simvastatin	
	Insulin-sensitive subjects (n = 21)	Insulin-resistant subjects (n = 22)	Insulin-sensitive subjects (n = 23)	Insulin-resistant subjects (n = 21)	Insulin-sensitive subjects (n = 20)	Insulin-resistant subjects (n = 22)
Homeostasis model assessment index						
Baseline	1.4 ± 0.3	4.3 ± 0.4^{c}	1.3 ± 0.2	4.5 ± 0.5^{c}	1.2 ± 0.3	4.6 ± 0.4^{c}
After 4 weeks	1.3 ± 0.2 (−7)	3.7 ± 0.4 (−14)^{c}	1.4 ± 0.3 (−8)	4.7 ± 0.5 (4)^{c}	1.2 ± 0.2 (0)	3.8 ± 0.4 (−17)^{c}
After 12 weeks	1.3 ± 0.3 (−7)	3.5 ± 0.5 (−19)^{c}	1.3 ± 0.3 (0)	4.6 ± 0.4 (2)^{c}	1.1 ± 0.2 (−8)	3.7 ± 0.4 (−19)^{c}
hsCRP (mg L^{-1})						
Baseline	2.9 ± 0.4	3.8 ± 0.3^{a}	3.0 ± 0.2	3.7 ± 0.3^{b}	2.9 ± 0.3	4.0 ± 0.3^{c}
After 4 weeks	2.6 ± 0.4 (−10)	2.9 ± 0.3 (−24)^{d}	2.1 ± 0.2 (−30)^{f,g}	2.8 ± 0.3 (−24)^{a,d}	2.2 ± 0.2 (−24)^{d}	2.0 ± 0.3 (−50)^{f,h,k}
After 12 weeks	2.8 ± 0.3 (−3)	2.5 ± 0.2 (−34)^{f,o}	1.6 ± 0.2 (−47)^{f,i,p}	2.1 ± 0.2 (−43)^{a,f,r}	1.5 ± 0.2 (−48)^{f,i,s}	0.9 ± 0.2 (−78)^{b,f,i,l,o,s}
ICAM-1 (ng mL^{-1})						
Baseline	250 ± 27	358 ± 34^{c}	246 ± 29	374 ± 39^{c}	242 ± 20	351 ± 33^{c}
After 4 weeks	239 ± 24 (−4)	266 ± 26 (−26)^{e}	191 ± 14 (−22)^{d,g}	285 ± 24 (−24)^{c,d}	193 ± 20 (−20)^{d}	210 ± 23 (−40)^{f,j}
After 12 weeks	240 ± 26 (−4)	254 ± 27 (−29)^{f,n}	156 ± 12 (−37)^{f,i,p}	240 ± 14 (−36)^{c,f,p}	162 ± 14 (−33)^{f,i}	133 ± 12 (−62)^{a,f,i,l,o,s}
TNF-α release (pg mL^{-1})						
Baseline	297 ± 32	422 ± 39^{c}	304 ± 22	430 ± 41^{c}	289 ± 25	423 ± 42^{c}
After 4 weeks	284 ± 30 (−4)	325 ± 40 (−23)^{d}	240 ± 20 (−21)^{d}	324 ± 26 (−25)^{b,e}	228 ± 15 (−21)^{d,g}	253 ± 24 (−40)^{f,i,l}
After 12 weeks	297 ± 41 (0)	299 ± 46 (−29)^{f,o}	193 ± 16 (−37)^{f,i,p}	250 ± 26 (−42)^{b,f,p}	186 ± 14 (−36)^{f,i,p}	165 ± 11 (−61)^{f,i,l,o,s}
IFN-γ release (ng mL^{-1})						
Baseline	46.5 ± 5.7	61.5 ± 5.2^{a}	47.1 ± 4.8	62.4 ± 5.1^{c}	46.0 ± 5.9	59.2 ± 6.9^{b}
After 4 weeks	43.0 ± 4.3 (−8)	48.0 ± 4.5 (−22)^{d}	35.9 ± 2.9 (−23)^{e,h}	47.8 ± 3.2 (−23)^{c,d}	33.8 ± 3.6 (−26)^{e,g}	39.2 ± 4.9 (−34)^{f}
After 12 weeks	43.2 ± 5.2 (−7)	45.7 ± 4.0 (−26)^{e,m}	25.6 ± 2.4 (−46)^{f,i,r}	40.5 ± 3.1 (−35)^{c,f,r}	27.6 ± 2.2 (−40)^{f,i,p}	22.3 ± 2.1 (−62)^{a,f,i,l,o,s}
IL-2 release (ng mL^{-1})						
Baseline	4.8 ± 0.5	6.3 ± 0.5^{b}	5.0 ± 0.4	6.5 ± 0.4^{b}	4.9 ± 0.3	6.4 ± 0.5^{b}
After 4 weeks	4.4 ± 0.5 (−8)	4.8 ± 0.5 (−24)^{d}	3.9 ± 0.3 (−22)^{d}	5.1 ± 0.3 (−22)^{c,d}	3.9 ± 0.4 (−20)^{e}	4.0 ± 0.2 (−38)^{f,g,l}
After 12 weeks	4.4 ± 0.5 (−8)	4.5 ± 0.5 (−29)^{e,m}	3.2 ± 0.4 (−36)^{f,h,p}	4.4 ± 0.2 (−32)^{b,f,r}	3.3 ± 0.3 (−33)^{f,h}	2.6 ± 0.4 (−59)^{f,i,l,o,s}

Each value represents the mean ± SD. Values in parentheses represent percentage changes from baseline values. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ vs. insulin-sensitive patients in the same treatment group. $^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.001$ vs. pretreatment values. $^{g}P < 0.05$, $^{h}P < 0.01$, $^{i}P < 0.001$ vs. the effect of ezetimibe in the same subgroup of patients. $^{j}P < 0.05$, $^{k}P < 0.01$, $^{l}P < 0.001$ vs. the effect of simvastatin in the same subgroup of patients. $^{m}P < 0.05$, $^{n}P < 0.01$, $^{o}P < 0.001$ vs. the effect of 12-week treatment with the same drug(s) in the other subgroup of patients. $^{p}P < 0.05$, $^{r}P < 0.01$, $^{s}P < 0.001$ vs. the effect after 4 weeks of treatment.

*Only data from subjects who completed the study were included in the final analyses. hsCRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule 1.

and ezetimibe [15] were found to reduce the number of T cells, the accurate procedure for lymphocyte isolation (including the same number of cells in each sample) enabled us to exclude the possibility that the observed decrease in cytokine release is secondary to the reduction in the number of these cells. Because T lymphocytes are one of the most important cells involved in atherogenesis [3, 4], and TNF-α, IFN-γ and IL-2 exhibit pro-atherogenic actions [20, 21], the results suggest that statin treatment offers more benefits than ezetimibe to patients with isolated hypercholesterolaemia, despite similar hypolipidaemic effects of these drugs. Considering the strong relationship between plasma hsCRP and the presence, onset and severity of atherosclerosis [22], as well as the established role of the assessed cytokines in the development and progression of atherosclerosis [20, 21], it seems that a weak anti-inflammatory effect of ezetimibe may bring some additional clinical benefits to patients with hypercholesterolaemia in whom statin therapy either is contraindicated or results in adverse effects. However, in the light of recent studies, which demonstrated a lack of benefit of ezetimibe on intima–media thickness in statin-treated subjects with either heterozygous familial hypercholesterolaemia [23] or type 2 diabetes [24], longer-term prospective controlled trials using more hard end-points in large patient groups are required to confirm this hypothesis.

The most interesting result of our study may be that ezetimibe potentiated a lymphocyte-suppressing effect of simvastatin. Moreover, the combined administration of simvastatin and ezetimibe was the only treatment that led to a small but statistically significant increase in HDL cholesterol and apoprotein A-I levels. These findings may suggest the superiority of the ezetimibe/statin combination compared with the effect of a statin alone and partially explain why ezetimibe administered together with simvastatin reduced the need for coronary artery bypass grafting in patients with aortic stenosis [25]. According to current recommendations, administration of ezetimibe should be considered if statin therapy is contraindicated, results in adverse effects or if statin monotherapy does not correct all lipid/lipoprotein abnormalities [26]. Our results may indicate that the combined treatment is justifiable even in subjects with relatively mild hypercholesterolaemia if they are at high cardiovascular risk. Because the combined treatment was well tolerated when the two agents were started simultaneously, it seems that hypercholesterolaemic individuals may be administered both ezetimibe and a statin from the beginning of treatment.

In the present study, we did not find any correlation between the degree of reduction in cytokine levels and the extent of lipid-lowering action of the drugs studied. In the case of simvastatin, an anti-inflammatory effect probably results from diminished post-translational protein prenylation, which is an important process for cellular signalling, differentiation and growth regulation, and membrane transport [27, 28]. It is more difficult to establish the molecular mechanisms responsible for a weak anti-inflammatory effect of ezetimibe. One of the potential signal transmission pathways may be aminopeptidase N, which is one of the molecular targets of ezetimibe [29]. This protein is expressed by monocytes/macrophages [13], which are in close proximity to lymphocytes within the atherosclerotic plaque [1, 2]. In line with this hypothesis, synthetic inhibitors of aminopeptidase N were found to suppress cytokine production by activated human T cells [30].

The ezetimibe-induced reduction in the level of ICAM-1 indicates an endothelial protective effect of ezetimibe. This is in agreement with the results of Kuhlencordt *et al.* [31] who found that ezetimibe potently reduced vascular expression of vascular cell adhesion molecule-1 in atherosclerosis-prone mice. The existence of a correlation between plasma levels of soluble ICAM-1 and hsCRP as well as between lymphocyte cytokine release and plasma hsCRP suggests that both enhanced lymphocyte cytokine release and endothelial dysfunction contribute to the development of systemic low-grade inflammation. Moreover, a reduction in TNF-α, IFN-γ, IL-2 and ICAM-1 production is in part responsible for the systemic anti-inflammatory effect of ezetimibe and simvastatin. As our results show, abnormal secretory function of T lymphocytes and endothelial dysfunction independently induce systemic inflammation, whereas treatment-induced reduction in plasma levels of hsCRP seems to result from the combined actions of statins and/or ezetimibe on lymphocytes and the endothelium.

Another interesting finding of our study is that ezetimibe, but not simvastatin, affected cytokine release and plasma hsCRP with a potency determined by the degree of insulin sensitivity. In subjects with normal sensitivity to insulin, ezetimibe had no anti-inflammatory effects, whereas the effect was almost as strong as that produced by simvastatin in insulin-resistant individuals. Our observation, the first to show that the anti-inflammatory effect of ezetimibe depends on insulin sensitivity, suggests that this agent should be administered in particular to isolated

hypercholesterolaemic patients with abnormal insulin sensitivity. In turn, in the case of simvastatin, the degree of insulin sensitivity seems to be of little importance for its action on lymphocytes and hsCRP.

Although both 4- and 12-week treatments with ezetimibe and simvastatin resulted in almost the same lipid-lowering effects, their effects on cytokine release and plasma levels of ICAM-1 and hsCRP were more pronounced at the end of the study protocol. This observation, suggesting that the pleiotropic effects of ezetimibe are time dependent, seems to justify the need for long-term treatment with statins, even if such therapy is not associated with any further lipid-lowering effects. It also raises the question of whether a longer period of treatment might bring any additional benefits compared with 12 weeks of therapy. We intend to investigate this in our future studies.

Our study has some limitations. Although the study was powered and the population exceeded the required number of individuals, our sample size was relatively small. As several differences in lymphocyte-suppressing, global anti-inflammatory and endothelial protective effects (particularly in the group treated with ezetimibe) did not achieve a *P*-value below 0.05 (ranging between 0.05 and 0.1), it appears that slightly larger groups would ensure significant differences. Moreover, the doses of ezetimibe and simvastatin were not maximal, and the duration of treatment was short. It is likely that the effect of both these agents is more pronounced in the case of long-term treatment with higher doses. In our study, the term 'insulin resistant' encompassed subjects with impaired fasting glucose, impaired glucose tolerance and some with normal glucose tolerance. We cannot exclude the possibility that the effect of ezetimibe and simvastatin may differ between the three subgroups. Because patients with diabetes were excluded, it remains unknown whether similar effects of ezetimibe and simvastatin are also observed in subjects with advanced glucose homeostasis abnormalities.

In conclusion, the results of our study demonstrate that simvastatin and, to a lesser extent, ezetimibe reduce lymphocyte cytokine release, reduce systemic inflammation and improve endothelial function. For ezetimibe, these actions were more evident in insulin-resistant than in insulin-sensitive patients. These effects, which are lipid-independent and more potent if both agents are administered together, may delay the onset and progression of atherosclerosis and related disorders.

Conflict of interests statement

None of the authors has any conflicts of interest to declare.

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References

1 Libby P, Okamoto Y, Rocha VZ, Folco E. Inflammation in atherosclerosis: transition from theory to practice. *Circ J* 2010; **74:** 213–20.
2 Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. *Annu Rev Immunol* 2009; **27:** 165–97.
3 Hansson GK. Inflammatory mechanisms in atherosclerosis. *J Thromb Haemost* 2009; **7(Suppl. 1):** 328–31.
4 Weyand CM, Younge BR, Goronzy JJ. T cells in arteritis and atherosclerosis. *Curr Opin Lipidol* 2008; **19:** 469–77.
5 Filippatos TD, Mikhailidis DP. Lipid-lowering drugs acting at the level of the gastrointestinal tract. *Curr Pharm Des* 2009; **15:** 490–516.
6 Miura S, Saku K. Ezetimibe, a selective inhibitor of the transport of cholesterol. *Intern Med* 2008; **47:** 1165–70.
7 Yagi S, Akaike M, Aihara K *et al.* Ezetimibe ameliorates metabolic disorders and microalbuminuria in patients with hypercholesterolemia. *J Atheroscler Thromb* 2010; **17:** 173–80.
8 Chan DC, Watts GF, Gan SK *et al.* Effect of ezetimibe on hepatic fat, inflammatory markers, and apolipoprotein B-100 kinetics in insulin-resistant obese subjects on a weight loss diet. *Diabetes Care* 2010; **33:** 1134–9.
9 Alvarez-Sala LA, Cachofeiro V, Masana L *et al.* Effects of fluvastatin extended-release (80 mg) alone and in combination with ezetimibe (10 mg) on low-density lipoprotein cholesterol and inflammatory parameters in patients with primary hypercholesterolemia: a 12-week, multicenter, randomized, open-label, parallel-group study. *Clin Ther* 2008; **30:** 84–97.
10 Kater AL, Batista MC, Ferreira SR. Synergistic effect of simvastatin and ezetimibe on lipid and pro-inflammatory profiles in prediabetic subjects. *Diabetol Metab Syndr* 2010; **2:** 34.
11 Gupta M, Szmitko PE, Tsigoulis M *et al.* Effects of ezetimibe add-on to statin therapy on adipokine production in patients with metabolic syndrome and stable vascular disease. *J Cardiovasc Pharmacol* 2010; **56:** 241–5.
12 Seedorf U, Engel T, Lueken A *et al.* Cholesterol absorption inhibitor ezetimibe blocks uptake of oxidized LDL in human macrophages. *Biochem Biophys Res Commun* 2004; **320:** 1337–41.
13 Orsó E, Werner T, Wolf Z *et al.* Ezetimib influences the expression of raft-associated antigens in human monocytes. *Cytometry A* 2006; **69:** 206–8.
14 Gómez-Garre D, Muñoz-Pacheco P, González-Rubio ML, Aragoncillo P, Granados R, Fernández-Cruz A. Ezetimibe reduces plaque inflammation in a rabbit model of atherosclerosis and inhibits monocyte migration in addition to its lipid-lowering effect. *Br J Pharmacol* 2009; **156:** 1218–27.

15 Shaw SM, Najam O, Khan U, Yonan N, Williams SG, Fildes JE. Ezetimibe and atorvastatin both immunoregulate CD4+ T cells from cardiac transplant recipients in vitro. *Transpl Immunol* 2009; **21:** 179–82.
16 Okopien B, Krysiak R, Kowalski J *et al.* The effect of statins and fibrates on interferon-gamma and interleukin-2 release in patients with primary type II dyslipidemia *Atherosclerosis* 2004; **176:** 327–35.
17 Krysiak R, Okopien B. The effect of simvastatin on lymphocyte secretory function in patients with impaired fasting glucose. *J Cardiovasc Pharmacol* 2010; **56:** 491–7.
18 Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20 536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002; **360:** 7–22.
19 Olsson AG, Schwartz GG, Jonasson L, Linderfalk C. Are early clinical effects of cholesterol lowering mediated through effects on inflammation? *Acta Physiol Scand* 2002; **176:** 147–50.
20 Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev* 2006; **86:** 515–81.
21 Schroecksnadel K, Frick B, Winkler C, Fuchs D. Crucial role of interferon-gamma and stimulated macrophages in cardiovascular disease. *Curr Vasc Pharmacol* 2006; **4:** 205–13.
22 Ridker PM. Inflammatory biomarkers and risks of myocardial infarction, stroke, diabetes, and total mortality: implications for longevity. *Nutr Rev* 2007; **65(12 Pt 2):** S253–9.
23 Kastelein JJ, Akdim F, Stroes ES *et al.* Simvastatin with or without ezetimibe in familial hypercholesterolemia. *N Engl J Med* 2008; **358:** 1431–43.
24 Fleg JL, Mete M, Howard JG *et al.* Effect of statins alone versus statins plus ezetimibe on carotid atherosclerosis in type 2 diabetes: the SANDS (Stop Atherosclerosis in Native Diabetics Study) trial. *J Am Coll Cardiol* 2008; **52:** 2198–205.
25 Rossebo AB, Pedersen TR, Boman K *et al.* Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis. *N Engl J Med* 2008; **359:** 1343–56.
26 Davidson H. Novel nonstatin strategies to lower low-density lipoprotein cholesterol. *Curr Atheroscler Rep* 2009; **11:** 67–70.
27 Krysiak R, Okopień B, Herman ZS. Effects of HMG-CoA reductase inhibitors on coagulation and fibrinolysis processes. *Drugs* 2003; **63:** 1821–54.
28 Jasinska M, Owczarek J, Orszulak-Michalak D. Statins: a new insight into their mechanisms of action and consequent pleiotropic effects. *Pharmacol Rep* 2007; **59:** 483–99.
29 Kramer W, Girbig F, Corsiero D *et al.* Aminopeptidase N (CD13) is a molecular target of the cholesterol absorption inhibitor ezetimibe in the enterocyte brush border membrane. *J Biol Chem* 2005; **280:** 1306–20.
30 Reinhold D, Bank U, Täger M *et al.* DP IV/CD26, APN/CD13 and related enzymes as regulators of T cell immunity: implications for experimental encephalomyelitis and multiple sclerosis. *Front Biosci* 2008; **13:** 2356–63.
31 Kuhlencordt PJ, Padmapriya P, Rütze S *et al.* Ezetimibe potently reduces vascular inflammation and arteriosclerosis in eNOS-deficient ApoEko mice. *Atherosclerosis* 2009; **202:** 48–57.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Exclusion criteria and lymphocyte cultures.

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