Statins as Immunomodulatory Agents

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Abstract—Statins have long been thought to exert their benefits by reducing cholesterol synthesis. However, the fact that mevalonate is the precursor of isoprenoids that regulate diverse cellular functions has led investigators to examine pleiotropic effects for these agents. Major histocompatibility complex class II (MHC-II) molecules, which affect the immune response and organ rejection after transplantation, may be induced by the proinflammatory cytokine interferon gamma (IFN-γ). An experiment was conducted to determine whether statins affect the regulation of MHC-II expression by IFN-γ in cultured human endothelial cells and monocyte/macrophages. Statins were found to repress the induction of MHC-II by IFN-γ. This may explain the immunosuppressive effects of statins seen in two clinical trials of organ transplantation and suggest a potential role for statins as immunosuppressive agents. (Circulation. 2004;109[suppl II]:II-15–II-17.)

Key Words: statins ■ T lymphocyte ■ macrophages ■ immunology

In the last decades, substantial progress has been made in understanding the relationship between lipid disorders and the prevention of cardiac ischemic disease.1 The identification of new therapeutic targets and new lipid-modifying agents expands treatment options. 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, have been described as the principal and most effective class of drugs to reduce serum cholesterol levels and have been shown to significantly reduce cardiovascular events and mortality in patients with or without coronary artery disease.2–6 The clinically beneficial effects of statins are usually assumed to result from their ability to reduce cholesterol synthesis.7 However, because mevalonate, the product of the enzyme reaction, is the precursor not only of cholesterol but also of many nonsterol isoprenoid compounds, inhibition of HMG-CoA reductase may produce pleiotropic effects.7–11

Indeed, the mevalonate pathway yields a series of isoprenoids that are vital for diverse cellular functions.8 These isoprenoids include: isopentenyl adenosine, present in some types of transfer of RNA; dolichols, required for glycoprotein synthesis; and polyisoprenoid side chains of ubiquinone and heme A, involved in electron transport. Several proteins have also been identified that are post-translationally modified by the covalent attachment of mevalonate-derived isoprenoid groups—either farnesyl or geranylgeranyl pyrophosphate.7,12 These proteins must be prenylated as a prerequisite for membrane association, which is required for their function. Members of this family are involved in a number of cellular processes, including cell signaling, cell differentiation and proliferation, myelination, cytoskeleton dynamics, and endocytic/exocytic transport.7

Hence, through the inhibition of HMG-CoA reductase, statins may affect a variety of processes; this may help to explain their nonlipid-related pharmacologic properties. Indeed, several recent in vitro and in vivo experiments have demonstrated that HMG-CoA reductase inhibitors have antiatherosclerotic effects that are not related to lipid lowering. Because of their broad and extensive clinically beneficial effects and their extremely low incidence of side effects, statins might even be seen as the new aspirin.13

Statins have never been shown to be involved in the immune response, although two clinical trials have suggested that, in heart transplant patients, statin therapy has beneficial effects on the incidence of cardiac rejection, coronary vasculopathy, and survival.14,15 To date, however, there has been no scientific rationale tested and proved in vitro that explains these beneficial effects of statins on the immune system. Major histocompatibility complex class II (MHC-II) molecules, expressed on the surface of specialized cells, are directly involved in the control of the immune response and thus determine rejection after organ transplantation. Whereas a limited number of specialized cell types express MHC-II constitutively, numerous other cells become MHC-II positive on induction by the inflammatory mediator interferon gamma (IFN-γ). This complex regulation is under the control of the transactivator CIITA, the expression of which is tightly regulated by several alternative promoters, operating under distinct physiological conditions.16–18 CIITA promoter IV is specifically responsible for the IFN-γ inducible expression of CIITA, and thus of MHC-II.18 We hypothesized that statins may regulate IFN-γ–induced MHC-II expression on antigen-presenting cells and in this way reduce T-lymphocyte activation.

Our laboratory used human vascular endothelial cells (ECs) and human monocytes/macrophages (MMs) that were stimulated with
human recombinant IFN-γ in the presence or absence of atorvastatin, lovastatin, pravastatin, or simvastatin. To detect MHC-II class expression, we used flow cytometry and immunohistochemistry labeling. To analyze CIITA regulation, we performed RNase protection assays and transfection experiments. To determine the functional effects of statins on MHC-II expression, we performed mixed lymphocyte reactions and measured T-lymphocyte proliferation by [3H]thymidine incorporation and IL-2 production (ELISA). We also studied the effect of several statins on the regulation of inducible MHC-II expression by IFN-γ in primary cultures of human ECs and MM.

These analyses led us to the following conclusions: (1) Statins effectively repress the induction of MHC-II expression by IFN-γ in a dose-dependent manner (Figure 1). (2) In the presence of L-mevalonate, the effect of statins on MHC-II expression is abolished, indicating that it is, in fact, as HMG-CoA reductase inhibitors that statins mediate repression of MHC-II. (3) Repression of MHC-II expression by statins is highly specific for the inducible form of MHC-II expression and does not affect constitutive expression of MHC-II in highly specialized antigen-presenting cells, such as dendritic cells and B lymphocytes. (4) This effect of statins is specific for MHC-II and does not affect MHC class I expression. (5) Pretreatment of ECs with statins reduces subsequent T-lymphocyte proliferation (mixed lymphocyte reactions), as measured by [3H]thymidine incorporation and IL-2 release (Figure 2). (6) In statin-treated samples, repression of induction of MHC-II by IFN-γ is paralleled by a reduced induction of CIITA mRNA by IFN-γ. Interestingly, the degrees of repression of CIITA mRNA induction observed with the different statins are reflected in the levels of repression of MHC-II expression observed with the same drugs. Constitutive expression of MHC-II, known to be mediated by CIITA promoters I and III, is not affected by statins. The specificity of statins for repressing inducible and not constitutive MHC-II expression suggests an effect on CIITA promoter IV. Indeed, we show that induction of expression of the first exon specifically controlled by CIITA promoter IV is affected by statins. Finally, (7) the STAT effect is transcriptional, as demonstrated by actinomycin D experiments used to block de novo RNA synthesis and explore mRNA half-life. That this effect is direct and does not require de novo protein synthesis may be seen by a lack of effect in experiments using cycloheximide.

All these effects of statins on MHC-II induction have been observed with the statins currently used in clinical medicine. Other investigators have confirmed these results. Future studies will tell us whether there is a link between mevalonate blockade due to statins and either the availability or the cooperative binding of the 3 well-defined transcription factors, Stat1, USF-1, and IRF-1, which are required for the activity of CIITA promoter IV. Other mechanisms unrelated to the HMG-CoA pathway may also be involved. Indeed, recent findings have demonstrated that several statins selectively inhibit LFA-1-mediated adhesion and costimulation of lymphocytes, and thus suppress inflammatory response in vivo.

More recently, our laboratory also demonstrated that statin treatment reduced the expression of CD40 on atheroma-associated cells in vitro, as well as on atherosclerotic lesions in situ in patients treated with statins. These results have been confirmed by other investigators. Since CD40 and its ligand CD154 have been implicated in several crucial immunologic pathways, these latest results, together with observation of the reduced MHC-II expression, provide even more evidence that statins have the potential to modulate the immune system.

Until now, clinical observations suggesting a beneficial effect of statin treatment on outcomes in heart transplantation have remained unexplained, because statins have never before been connected to the immune system. We have discovered a novel effect of statins as an effective repressor of MHC-II

Figure 1. Atorvastatin decreased IFN-γ-induced MHC-II protein expression on human endothelial cells. Each panel is a histogram representing cell numbers (y axis) versus log fluorescence intensity (x axis) for 30,000 viable cells. Flow cytometry analysis for MHC-II. A, Human vascular ECs untreated (gray line) or treated with IFN-γ (500 U/mL, 48 hours) alone (solid histogram). B, ECs treated with IFN-γ (500 U/mL, 48 hours) alone (solid histogram) or with atorvastatin, either (1) 225 nmol/L, (2) 75 nmol/L, or (3) 25 nmol/L. Similar results were obtained in independent experiments with ECs from 6 different donors. From Kwak et al.

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expression, and we have provided a detailed molecular explanation for this unexpected and novel effect of one of the most intensely used drugs in medicine. Because statin-induced repression of MHC-II also represses MHC-II–dependent activation of T lymphocytes, statins likely have an immunosuppressive effect. This discovery provides a firm scientific rationale for suggesting the use of this drug as an immunosuppressor in organ transplantation. It also suggests numerous other practical clinical applications of statins as novel immunomodulators, particularly in diseases in which aberrant expression of MHC-II and CD40 and/or aberrant activation of CD4 T lymphocytes are implicated. Beyond organ transplantation, these range from various autoimmune diseases, including type 1 diabetes, multiple sclerosis, and rheumatoid arthritis, to chronic inflammatory diseases such as atherosclerosis. The high degree of patient tolerance of statins makes them potentially a welcome addition to the limited current arsenal of immunosuppressive agents. In vivo studies will be necessary to confirm such an effect.

A major challenge of contemporary medicine is to break the traditional compartmentalization that frequently separates different fields. This is true as well for basic biochemical mechanisms and the practice of clinical medicine. Unexpected linkages between different areas of medicine may turn out to be of great interest. An unsuspected “bridge” between molecular immunology and cardiology practice, as presented here, is a good example of such a linkage.

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Figure 2. Functional consequences of inhibition of major histocompatibility complex class II antigens by statins on T lymphocyte activation. A, [3H]Thymidine incorporation measured in allogenic T lymphocytes exposed (5 days) to human ECs pretreated for 48 hours with (1) IFN-γ (500 U/mL) alone, or (2) IFN-γ (500 U/mL) with atorvastatin (10 μmol/L). Similar results were obtained in independent experiments with ECs from three different donors (P<0.02 compared with IFN-γ–treated cells). B, Interleukin-2 (IL-2) release measured by enzyme-linked immunosorbent assay in supernatants of allogenic T lymphocytes exposed (48 hours) to human ECs pretreated during 48 hours with (1) IFN-γ (500 U/mL) alone, or (2) IFN-γ (500 U/mL) with atorvastatin (10 μmol/L). Similar results were obtained in independent experiments with ECs from four different donors (P<0.01 compared with IFN-γ–treated cells). From Kwak et al.19

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