

Statins modulate the murine immune response and enhance graft longevity in human kidney transplant recipients

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Abstract—Multiple immunomodulatory effects have been described for statins. We sought to assess their effects on components of the immune response in mice in addition to examining their effects in human transplant recipients. Multiple groups of BALB/c mice were challenged with egg albumin and treated with various regimen of atorvastatin. Serum and spleen samples were then collected. Mice sera were analyzed for anti-egg albumin levels by enzyme linked immunosorbent assay (ELISA) while splenocytes were assessed for IL-4 and IFN- γ expression by reverse transcriptase polymerase chain reaction (RT-PCR). Atorvastatin treatment markedly decreased anti-egg albumin antibody, IL-4 and IFN- γ production particularly in a group of mice receiving atorvastatin for 5 days post-challenge. To examine the effect of statins on graft longevity in kidney allograft recipients, 111 patients were monitored for graft rejection or loss within a period of 3 years post-transplantation. Fifteen of 90 (16.66%) of patients who were not on a statin had a rejection episode or lost the graft while 1 of 21 (4.76%) patients on a statin had a rejection episode. Therefore, statins appear to have suppressive effects on various components of the immune response. They also seem to decrease the risk of graft rejection or loss in transplant recipients.

I. INTRODUCTION

Statins are ubiquitously prescribed agents used for the treatment of hyperlipidemia, in particular for decreasing blood cholesterol levels. They are 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors that inhibit mevalonate biosynthesis, the rate-limiting step of cholesterol production [1, 2]. They comprise a group of compounds that are natural or semisynthetic derivatives of fungal metabolites in addition to those that are purely synthetic [3]. A decrease in the synthesis of cholesterol results in upregulated expression of hepatic LDL (low-density lipoprotein) receptors and consequential enhanced clearance of bloodstream LDL [4].

The role of statin therapy in reducing the risk of cardiovascular disease is generally well established [5-7]. This was initially attributed to the reduction of plasma cholesterol level; however, multiple studies have since shown that statins have several beneficial effects that extend beyond altering cholesterol metabolism. These pleiotropic effects of statins involve improvement of endothelial function, prevention of thrombosis, enhanced stability of atherosclerotic plaques, anti-oxidation, immunomodulation and abatement of the inflammatory reaction [8]. Some of the mechanisms behind these effects are directly related to inhibition of mevalonate synthesis and the resulting reduced production of non-sterol isoprenoid intermediates whereas other mechanisms are believed to be independent of the inhibition of cholesterol biosynthesis [9]. As a consequence of these pleiotropic effects, statins may be useful in a wide range of diseases other than hypercholesterolemia and heart-associated conditions. These include immune-mediated disorders such as rheumatoid arthritis [10] and multiple sclerosis [11]. They also have potential therapeutic applications in osteoporosis [12], cancer [13-15], cerebrovascular disorders [16], Alzheimer's disease and Parkinson's disease [11] among others. Further research and clinical trials are required to substantiate the use of statins in these conditions. In addition to the conditions mentioned above statins may also benefit transplant recipients [17, 18] owing to the immune mechanisms generally behind a rejection reaction or the loss of a grafted organ. We sought to assess the effect of statins on components of the immune response in mice in addition to examining the effects of these drugs on transplant recipients.

II. MATERIALS AND METHODS

Mouse groups and treatments

Four groups of animals each containing 9 female 8-week old female BALB/c mice were challenged with egg albumin (0.2 mg) via intraperitoneal injection. Mice received atorvastatin (Lipitor[®], Pfizer Inc., NY, USA) at a dose of 40 mg/Kg/mouse either directly after challenge, 24 hours before or 24 hours after challenge. One group received a daily atorvastatin dose up to 5 days after challenge. Two additional mouse groups served as control: one was challenged with egg albumin but was not treated with atorvastatin while the other received the sterile phosphate buffered saline (PBS) used to prepare all injections. Injection volumes did not exceed 0.5 ml per mouse per day. Three mice per group were euthanized on days 6 and 12 post immunization with egg albumin. Serum samples were collected and pooled upon cardiac puncture and spleen samples were also obtained from the euthanized animals. Approval for work utilizing mice was obtained from the Institutional Animal Care and Use Committee (IACUC) at the American University of Beirut.

Enzyme-linked immunosorbent assay (ELISA) for anti-egg albumin levels

Serum levels of anti-egg albumin were determined using ELISA. A 96 well polystyrene plate was coated with egg albumin by addition of 50 µl of a solution of egg albumin in PBS (25 µg/ml) into each well. This was followed by incubation at 4°C overnight. The plate was then washed 3 times with 300 µl of PBS per well using an automated washer (Bio-Tek[®] Instruments, VT). Subsequently 25 µl of test sera were added per well. Wells that did not include mouse sera were included as negative controls and used as blanks for antibody concentration calculation. This was followed by incubation at 37°C for 30 min; upon incubation the wells were washed as described above. Subsequently, 25 µl of a peroxidase-conjugated mouse anti-IgG in PBS (1 µg/ml) were added per well. This was followed by incubation at 37°C for 30 min; the plate was then washed as described above. Following this step 25 µl of 3,3',5,5' tetramethylbenzidine (TMB) were added per well and incubated for 30 min in the dark. Reactions were stopped with 25 µl of 3M NaOH per well. Absorbances were then read with a plate reader (Bio-Tek Instruments) at 450 nm.

Reverse transcriptase polymerase chain reaction (RT-PCR) for IL-4 and IFN-γ

RNA was isolated from mouse splenocytes using the TRIR[®] reagent (ABgene, UK) according to the manufacturer's instructions. cDNA synthesis was then carried out on the RNA samples using the Ready-to-Go[™] You-Prime-First-Strand Beads (Amersham Pharmacia Biotech, Piscataway, NJ) following the manufacturer's recommendations and using 100 ng of RNA per cDNA synthesis reaction. PCR was then carried out in 100 µl reactions containing 33 µl of the synthesized cDNA, 40 pmol of the forward primer, 40 pmol of the reverse primer and 2.5 units of Taq DNA polymerase. Previously published primers [19] were used for IL-4 transcript detection. These had the sequences: 5'-TCGCCATTTTGAACGAGGTC-3' and 5'-GAA AAG CCC GAA AGA GTC TC-3'. Previously published primers [19] were used for IFN-γ transcript detection. These had the sequences: 5'-AAAGAG ATA ATCTGGCTCTGC-3' and 5'-GCTCTGAGACAATGAACGCT-3'. PCR consisted of 35 cycles of 93°C for 2 min, 60°C for 1 min and 71°C for 2 min.

Kidney allograft recipients

One hundred and eleven kidney transplant recipients were studied. Of these, 32 were females and 79 were males. The mean age of the study population was 44.37 (Std. deviation: 15.33). Rejection or loss of the graft within a maximum period of 3 years following transplantation was monitored. Twenty one patients were on statins including 10 who were on simvastatin, 5 on atorvastatin, 4 on fluvastatin, 1 on pravastatin and 1 on rosuvastatin. Approval of the Institutional Review Board (IRB) at the American University of Beirut was obtained for the performed studies.

III. RESULTS

Effects of atorvastatin on the immune response in mice

To assess the effect of statins on the immune system, we examined responses in mice challenged with egg albumin and treated with atorvastatin as described in the Materials and Methods section. We observed a two-fold reduction in anti-egg albumin antibody levels in the group that received daily atorvastatin doses for 5 days when mouse sera were analyzed 6 days after challenge. This same group showed a four-fold reduction in antibody levels 12 days after challenge (Figure 1). Other treatments did not result in marked antibody level changes. Analysis of IL-4 and IFN-γ expression on RNA from splenocytes isolated from the mouse groups treated as explained above was then performed using RT-PCR. A sharp decline in detection of IL-4 expression was seen in the mouse group that received a daily dose of atorvastatin for 5 days when splenocytes were tested 6 days after egg albumin challenge. Expression of IL-4 was not detectable 12 days post-challenge in this group. On the other hand, IFN-γ expression was undetectable in this group on both days 6 and 12 post-challenge. Expression of these cytokines was detectable by RT-PCR in splenocytes from the other mouse groups [20].

Effect of statins on graft longevity

Fifteen of 90 (16.66%) kidney allograft recipients who were not on a statin had a rejection episode or lost the graft. On the other hand, only 1 of 21 (4.76%) recipients on a statin had a rejection episode. This patient was on fluvastatin.

IV. DISCUSSION

We intended to assess the effects of statins on the immune system in mice and on the graft longevity in kidney allograft recipients. These agents have lipid-lowering effects and are prescribed to transplant patients on immunosuppressive drugs that increase serum cholesterol levels. We observed suppression in antibody production upon antigenic challenge in mice in addition to suppression of IL-4 and IFN- γ expression. This supports previous observations by our group and others indicating that statins can simultaneously curb Th1 and Th2 immune responses [20-22]. Moreover, we have observed an up to four-fold decrease in the survival rate of *Candida albicans* or *Escherichia coli*-infected mice treated with atorvastatin. This was in comparison to infected mice that were not treated with this statin (manuscript in preparation). This indicates that the immunosuppressive effects of statins may curb immune responses required for combating infections.

On the other hand, we observed an approximate three-fold decrease in the risk of graft rejection in kidney graft recipients who were treated with a statin. We have previously ruled out other factors that may potentially affect graft longevity including whether the graft was obtained from a living related donor or an unrelated one; the degree of HLA-disparity also seemed to have no effect on graft longevity in our previous studies [23, 24]. Although we have previously shown that triple immunosuppressive therapy (with cyclosporine, mycophenolate mofetil and prednisone) seems to have a less protective effect on graft longevity compared to quadruple therapy (additionally includes sirolimus), the immunosuppressive regimen implemented appears to have no bearing on the effect of statins as we previously demonstrated [25]. Therefore, this protective effect of statins is seemingly independent of the immunosuppressive regimen administered.

Multiple mechanisms may contribute to the effects of statins we describe herein. These agents have been shown to inhibit signaling mediated by mitogen-activated protein kinase (MAPK) and peroxisome proliferator activated receptors (PPAR) [26] in addition to nuclear factor kappa B (NFkB) [27]. These pathways play a role in mediating inflammation. Statins also reduce the production of various cytokines [27-30] in addition to that of C-reactive protein (CRP) [31]. Moreover they seem to modulate the proliferation and activation of various types of cells with roles in immune pathways [32-36]. Additionally they decrease the expression of adhesion molecules [37, 38] and human leukocyte antigens (HLA) of class II type [39].

V. CONCLUSIONS

Statins appear to have clinical benefits in kidney transplant recipients. On one hand, they decrease cholesterol levels typically induced by the other immunosuppressive drugs administered; on the other, they considerably decrease the risk of graft rejection or loss. Further clinical studies should be performed to assess the utility of statins in various types of organ transplant recipients. Moreover, the optimum types of statins and their dosage regimen should be examined.

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Figure legends

Figure 1. Anti-egg albumin antibody levels in mice challenged with egg albumin and treated with atorvastatin. Six groups of mice were treated as indicated in the text and respective sera were analyzed by ELISA. The group that did not receive egg albumin or atorvastatin was administered sterile PBS instead. Antibody levels are normalized to those detected in the mouse group challenged with egg albumin but not treated with atorvastatin

Figure 1

