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Chapter 2

INTERLEUKIN-9 IN ATHEROSCLEROSIS: THERAPEUTIC APPLICATION AND ENDOGENOUS FUNCTION

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ABSTRACT

IL-9 is a pleiotropic cytokine with diverse biological effects. We determined the role of IL-9 on *de novo* atherosclerosis in LDLr deficient mice. Firstly, LDLr deficient mice were treated with recombinant IL-9 (1 µg/day/mouse; i.p.) for 5 weeks. In both female and male LDLr deficient mice, we observed a highly significant reduction in the extent of atherosclerosis of 59% ($p < 0.05$) and 66% ($p < 0.05$), respectively. To determine the role of endogenous IL-9 in atherogenesis, LDLr deficient mice were vaccinated against IL-9 and the induced anti-IL-9 antibodies functionally blocked endogenous IL-9. Blockade of IL-9 by vaccination led to a 2.4-fold increase in atherosclerosis in LDLr deficient mice. The observation that the expression of endogenous IL-9 rapidly decreased after a cholesterol rich diet in the spleen supports the protective role of endogenous IL-9 during atherosclerosis. IL-9 not only affected the production of pro-inflammatory cytokines but also affected two other hallmarks of atherosclerosis: β -VLDL induced foam cell formation in peritoneal macrophages was inhibited for 55% by IL-9, while the adhesion of monocytes to endothelial cells was also reduced. We conclude that endogenous IL-9 protected against atherosclerosis, while treatment with IL-9 reduced atherosclerosis. IL-9 and its receptor are therefore interesting new targets for the development of anti-inflammatory drugs for the treatment of atherosclerosis.

INTRODUCTION

Atherosclerosis is the main underlying pathology of cardiovascular disease and the formation of atherosclerotic plaques within affected arteries is to a large extent an inflammatory process characterized by the presence of macrophages, T cells and smooth muscle cells within the intima, by the formation of extra cellular matrix (ECM) and by the deposition of lipid^{1,2}. Both innate and adaptive immune responses play an important role in the process of atherosclerosis, which is reflected by a high production of pro-inflammatory cytokines within atherosclerotic plaques¹⁻³. The expression of pro-inflammatory cytokines is decisive for the initiation of atherosclerosis and for the progression of atherosclerotic lesions from fatty streak to fibrous plaque/unstable plaque¹⁻³. Pro-inflammatory cytokines, such as interleukin (IL)-1⁴, IL-6^{5,6}, IL-8⁷, IL-12^{8,9}, IL-15^{10,11}, IL-18¹²⁻¹⁴, and interferon (IFN)- γ ^{15,16} and various chemokines¹⁷ have specific pro-atherosclerotic effects during various stages of lesion¹⁸. A reduction in the expression of pro-inflammatory cytokines may, in addition to regulation of plasma lipid levels, be considered as one of the main targets for the treatment of atherosclerosis¹⁸.

A limited number of members of the cytokine family, such as IL-10 and IL-9, displays anti-inflammatory features¹⁸. These cytokines downregulate the production of pro-inflammatory cytokines by macrophages and T-cells during inflammatory responses¹⁹⁻²⁴. Interestingly, the endogenous expression of IL-10 in the human atherosclerotic plaque is accompanied by a low expression of pro-inflammatory markers (e.g. iNOS)²⁵, which may indicate an intrinsic, anti-atherosclerotic role for endogenous IL-10. The atheroprotective activity of IL-10 is further substantiated by studies with transgenic mice²⁶⁻²⁸, which show that IL-10 deficient mice are significantly more susceptible (2.5-30-fold) to atherosclerosis²⁶, whereas IL-10 overexpressing mice develop less atherosclerosis²⁸. We have shown that adenoviral IL-10 gene therapy in hyperlipidemic low-density lipoprotein (LDL) receptor (LDLr) deficient mice results in a prolonged expression of IL-10 in serum and a dramatic decrease in atherosclerotic lesion formation²⁹.

Some of the anti-inflammatory properties of IL-9 have also been revealed. IL-9 is produced by the Th2 subset of T-cells^{30,31} and IL-9 acts as a mild growth factor for certain T cell clones, B lymphocytes, mast cells, and some hematopoietic progenitor and lung epithelial cells³¹⁻³³. IL-9 has a beneficial effect on the expulsion of the nematode *Trichuris muris* and on a bacterial infection with *Pseudomonas aeruginosa*³⁴, but IL-9 is a susceptibility factor in *Leishmania major* infection³⁵, anaphylaxis³⁶ and may exert deleterious effects in the in asthma³⁷⁻⁴¹. These effects are underlined by studies with IL-9 overexpressing mice, which exhibit an increased susceptibility to lymphomagenesis⁴², intestinal mastocytosis⁴³, expansion of the B-1 lymphocyte population⁴⁴, bronchial hyperresponsiveness^{37,40} and airway eosinophilia⁴⁵, which indicate a link for IL-9 and its receptor to the susceptibility to asthma^{46,47}. Studies with IL-9 deficient mice show, however, that IL-9 is not mandatory for the induction of asthma.

Administration of IL-9 diminishes the response to gram-negative bacterial septic shock³¹. Pretreatment with IL-9 lowered the levels of TNF- α , IL-12 and IFN- γ after subsequent LPS challenge, whereas IL-9 treatment increased the level of IL-10. This decrease in IL-12 levels with IL-9 may be

beneficial in atherosclerosis, since the blockade of IL-12 attenuates atherosclerosis⁴⁸, while an increased expression of IL-10 is also beneficial for the outcome of atherosclerosis²⁶⁻²⁹.

We now show that prolonged treatment of LDLr deficient mice with IL-9 resulted in a more than 2-fold reduction in atherosclerosis, while vaccination against endogenous IL-9 results in a two-fold enhanced susceptibility of LDLr deficient mice to atherosclerosis. We conclude that IL-9 may play a, yet undiscovered, prominent role in the susceptibility towards atherosclerosis.

MATERIALS AND METHODS

Materials

Ovalbumin, hematoxylin, goat anti-rat IgG alkaline phosphatase conjugate, nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate, complete and incomplete Freund's adjuvant were from Sigma (St. Louis, Mo, USA). Western-type diet containing 0.25% cholesterol and 15% cocoa butter was from Special Diet Services (Witham, Essex, UK). Recombinant murine IL-9 was purified from baculovirus-infected Sf9 insect cell cultures, as described⁴⁹.

Surgery

LDLr deficient mice, 10–12 weeks old, were put on a Western-type diet two weeks before collar placement. Collars were prepared and inserted as described previously⁵⁰.

IL-9 treatment

Mice equipped with collars were treated with IL-9 starting by an intraperitoneal injection of 1 µg of IL-9 for 35 days.

IL-9 vaccination

IL-9-OVA complexes were obtained by cross linking mouse IL-9 and ovalbumin (Sigma) using glutaraldehyde³⁴. LDLr deficient mice were primed subcutaneously in the footpad with a 100 µl 1/1 mixture of CFA and complexed proteins in PBS. Three boosts were given with the same amount of antigen, mixed 1:1 with IFA, after 2, 4 and 6 wks. Control mice received an equivalent amount of OVA in Freund's adjuvant. Following the last boost, LDLr deficient mice were put on a Western-type diet for two weeks and collars were placed around the carotid arteries. Anti-IL-9 autoantibody titers were determined as described^{34,51}.

Tissue harvesting and histological analysis

Six weeks after collar placement, we obtained carotid artery specimens after *in situ* PBS perfusion for 15 minutes, followed by 10% neutral buffered formalin and analysed as morphometry was performed exactly as described⁴⁹.

Western-type diet, atherosclerosis and mRNA expression in the spleen

Total tissue RNA was isolated from LDLr deficient mice that had been fed a Western-type using the Guanidium-isothiocyanate (GTC) method⁵². RevertAid M-MuLV enzymatic kit (MBI) was used for RT reactions with 1 µg of total RNA as starting material. Resulting cDNA was dissolved in 400 µl, and 5 µl of cDNA was used in the Real Time PCR assay⁵². In the same animals we determined the extent of atherosclerosis in the aortic root as described⁵². Real time PCR assays were performed exactly as described using the $\Delta\Delta C_t$ method⁵².

Plasma analysis

Plasma fractionation was performed on a Superose 6 column by using a SMART HPLC system (Pharmacia, Uppsala, Sweden). Cholesterol and triglyceride content of total and fractionated serum were quantified colorimetrically (Roche Diagnostics).

***In vivo* TNF- α production assay**

LDLr deficient mice were injected intravenously with 20 µg/kg LPS from Salmonella minnesota R595 (List Biological Laboratories Inc) and at different time points TNF- α levels were determined by ELISA (OptEIA kit, Pharmingen).

***In vitro* foam cell formation**

Peritoneal macrophages were isolated by peritoneal lavage from LDLr deficient mice⁵³ and cultured for 24 hours in the presence of rat β -VLDL (100 µg/ml) with or without murine IL-9 (40 ng/ml). Thereafter, cells were fixated and the stained for lipid using oil red O (Sigma), followed by hematoxylin counterstaining and the lipid area was quantified using a Leica DMRE microscope and Leica Qwin Imaging software (Leica Ltd.).

Adhesion of monocytes to endothelial cells

Mouse endothelial (H5V) cells were grown on cover slips until monolayer, cells were subsequently pretreated for 16 hour with or without IL-9 and then activated with 50 ng/ml LPS⁵⁴ for 6 hours in the presence or absence of IL-9. Subsequently, the endothelial cells were labeled with calcein-AM and incubated for 30 minutes with Dil labeled RAW cells. Non-attached RAW cells were washed away and the number of RAW cells attached to the endothelial cells was determined using LEICA microscope.

Statistical analysis

Mann-Whitney nonparametric test and an unpaired student T test were performed to determine statistical significance. P values < 0.05 were considered significant.

RESULTS

Effect of IL-9 treatment on atherosclerosis

The effect of IL-9 on the TNF- α response after intravenous LPS triggering was determined in LDLr deficient mice. In line with the data of Grohmann *et al*³¹, we determined that an i.p. injection with rIL-9 (4 μ g/mouse) at 24 hrs and 1 hr before an intravenous injection of LPS (50 μ g/kg body weight) significantly reduced the plasma levels of TNF- α in LDLr deficient mice at 1 hour after LPS injection by 66 ± 5 % ($p < 0.05$, not shown).

Subsequently, we determined the effect of prolonged treatment with IL-9 on atherogenesis. Atherosclerosis was induced by placement of collars around the carotid artery of LDLr deficient mice that had been fed a Western-type diet (0.25% cholesterol) for 14 days. Seven days after collar placement, we started to treat LDLr deficient mice with a daily injection of IL-9 (1 μ g/mouse, i.p. injected). IL-9 treatment did not affect plasma cholesterol levels and lipoprotein profiles and **Fig. 1A/D** show that total plasma cholesterol levels were comparable in IL-9 treated and control treated LDLr deficient mice. In addition, analysis of the plasma distribution of cholesterol over the various lipoprotein fractions also showed no difference between the IL-9 and control treated group (data not shown). After 35 days of treatment, mice were sacrificed and the extent of atherosclerosis was determined in the carotid artery. The treatment of both male and female LDLr deficient mice resulted in a significant decrease in the extent of atherosclerosis (**Fig. 1B/E**). Control treated female mice developed more atherosclerosis than control treated male mice, but the effect of IL-9 was highly significant in both groups with an inhibition of atherosclerosis of 66% (male mice, $p=0.022$) and 59% (female mice, $p=0.032$), respectively, indicating that the relative reduction in atherosclerosis was comparable in male and female mice. In addition we observed a decrease in the intima to lumen ratio (**Fig. 1C/F**) upon treatment with IL-9, whereas the intima to media ratio was not affected. Analysis of the composition of the plaque showed no significant changes in plaque composition. The plaque was significantly smaller after treatment with IL-9, but in general the relative amount of macrophages, α smooth muscle actin-positive cells and collagen (on Masson's trichrome stain) did not differ significantly between the IL-9 treated and control treated mice (**Fig. 1G-J**). This may imply attenuation in the rate of atherogenesis, rather than an alteration in the phenotype of the plaque.

Neutralization of IL-9 and atherosclerosis

Next, to investigating the therapeutic role for IL-9, we aimed to identify the role of endogenous IL-9 in atherosclerosis. To that end, endogenous IL-9 was blocked using a vaccination protocol³⁴ and LDLr deficient mice were vaccinated against IL-9 using an IL-9-ovalbumin complex. IL-9 vaccination did not affect plasma lipid levels (**Fig. 2A**). Serum from vaccinated and control mice was tested for its ability to functionally block the action of IL-9 in a bioassay. All sera obtained from the LDLr deficient mice 14 days after the fourth round of vaccination displayed a high capacity to inhibit the proliferation of TS1 cells in response to IL-9, indicating that not only an antibody response against IL-9 was elicited by vaccination but also that the antibodies raised functionally blocked the action of IL-9 (**Fig. 2B**).

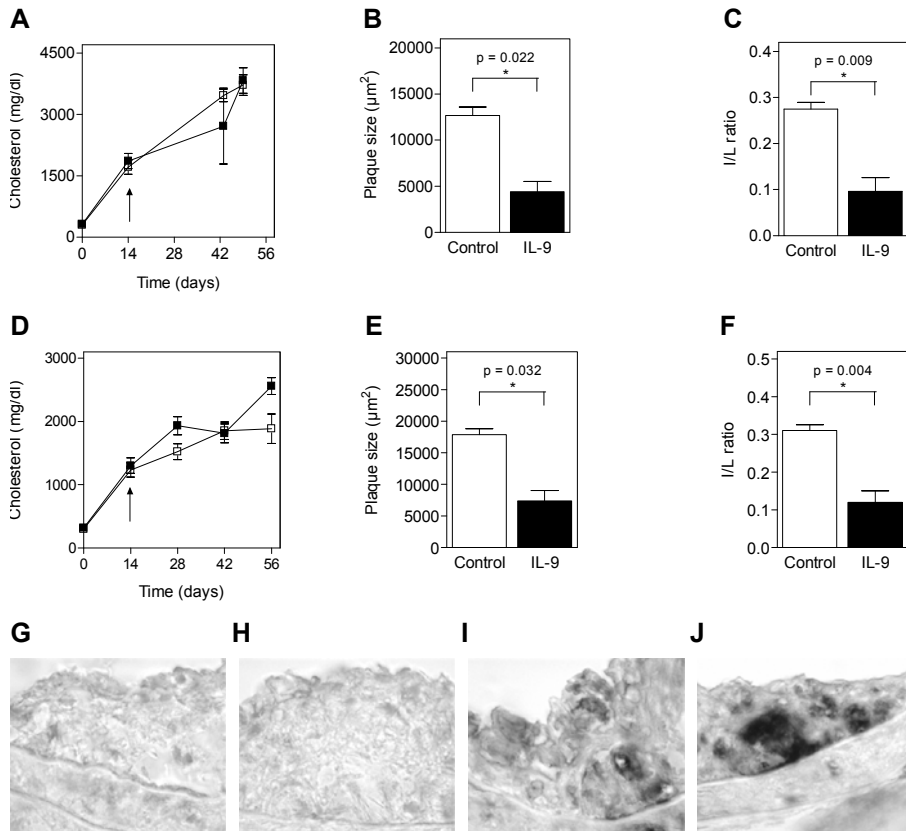


Fig. 1: Effect of IL-9 treatment on atherosclerosis. LDLr deficient mice were put on a Western-type diet and collars were placed around the carotid artery after 14 days. Male (A-C) and female (D-F) mice were treated from 7 days after collar placement until 42 days after collar placement with IL-9 (closed symbols, 1 µg/mouse/day, i.p. injection) or vehicle treated (open symbols, PBS containing 1% normal mouse serum). A,D Total plasma cholesterol levels were determined during the treatment. Mice were sacrificed and plaque size (B,E) as well as the intima/lumen ratio (C,F) was determined. Data are mean±SEM for the indicated number of animals. Plaque composition of control (G/I) and IL-9 treated (H/J) female LDLr deficient mice was determined using a macrophage (G, H) and a smooth muscle cell (I, J) specific staining. Representative sections from each group are shown.

The sera of mice that only received adjuvant displayed no IL-9 blocking activity or antibodies (Fig. 2B). After vaccination, both groups of mice were put on a Western-type diet, 14 days later collars were placed to induce atherosclerosis and 6 weeks thereafter mice were sacrificed to determine the extent of atherosclerosis. The blockade of endogenous IL-9 using vaccination resulted in a significant increase in the degree of atherosclerosis. IL-9 vaccinated LDLr deficient mice exhibited 2.4-fold more atherosclerosis than control vaccinated LDLr deficient mice, both with respect to plaque size and intima/lumen ratio (Fig. 2C/D). These data show that endogenous IL-9 is active in the LDLr deficient mice and is able to protect mice against diet induced atherosclerotic plaque formation. Relative plaque content of smooth muscle cells, macrophages and collagen did not differ between the groups as shown by (immuno)histochemical analysis of

the plaques (**Fig. 2E-J**). The plaques in the IL-9 vaccinated group, however, were found to be more advanced, with a large necrotic core underlying a well-demarcated fibrous cap.

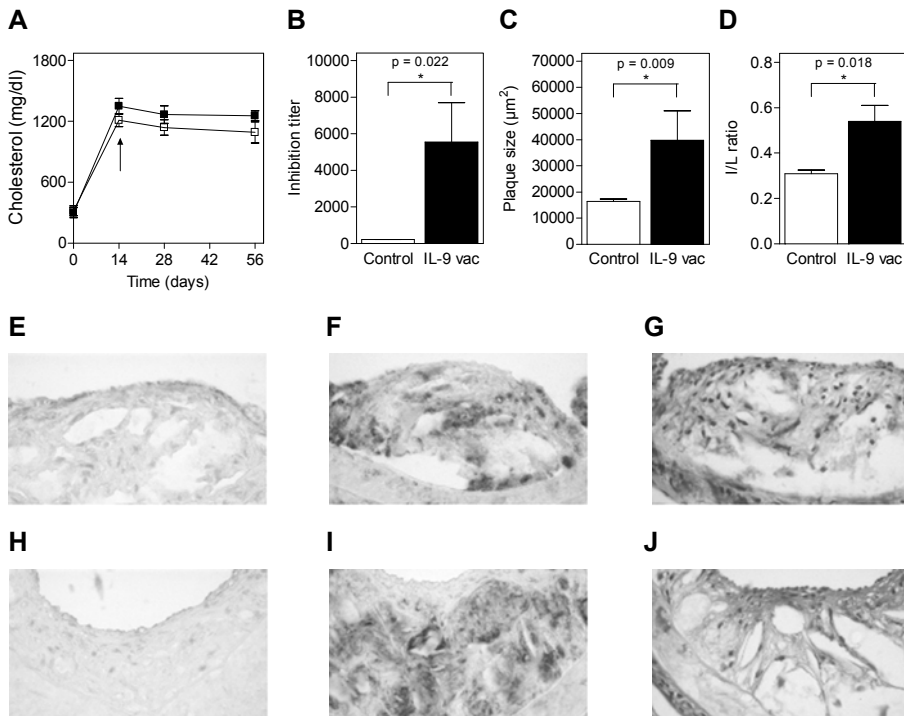


Fig. 2: Effect of vaccination against IL-9 on atherosclerosis. Female LDLr deficient mice were vaccinated against IL-9 using IL-9-ova complexes or vehicle. **A** Total plasma cholesterol levels were determined in IL-9 vaccinated (closed symbols) and control vaccinated mice (open symbols) during treatment. **B** The functional blockade of endogenous IL-9 was determined in a bioassay after 4 rounds of vaccination using IL-9-ova complexes or vehicle controls. **C/D** Atherosclerosis, induced by collar placement, was determined proximal to the collars six weeks after collar placement in IL-9 vaccinated and control vaccinated mice. In addition to determination of the plaque size, the intima/lumen ratio (I/L ratio) was determined. Data are mean \pm SEM (n=6-9). **E-J** Plaque composition of control treated (**E,F,G**) and IL-9 vaccinated (**H,I,J**) female LDLr deficient mice was determined using a macrophage (**E,H**), α -smooth muscle cell (**F, I**) and a collagen (**G,J**) specific staining. Representative sections are shown for each group.

Expression of IL-9 during atherosclerosis

The role of endogenous IL-9 in atherosclerosis was further established by determining the splenic expression of IL-9 during atherogenesis in LDLr deficient mice on Western-type diet. We determined cholesterol levels, lesion size in the aortic root and cytokine expression of two anti-inflammatory cytokines in the spleen, the largest immune organ in the body. Upon cholesterol feeding plasma cholesterol levels quickly rose and 42 days after the start of the diet initial lesions are found in the aortic root and their size increased rapidly thereafter (**Fig. 3A/B**). IL-10 is a well-known anti-inflammatory cytokine and the expression of IL-10 in T cells inhibits atherosclerosis. Data show that in parallel with the initiation of atherosclerosis IL-10 is 45.0% and 62.3% downregulated in the spleen at

63 and 84 days of feeding the Western-type diet (**Fig. 3C**). The IL-9 expression in the spleen also significantly decreased by 46.4%, 63.5% and 60.1% at 42, 63 and 84 days after the start of the diet, respectively (**Fig. 3C**). Interestingly, the decrease in splenic IL-9 expression preceded the decrease in IL-10 expression, suggesting a role for IL-9 in the regulation of IL-10 during atherogenesis.

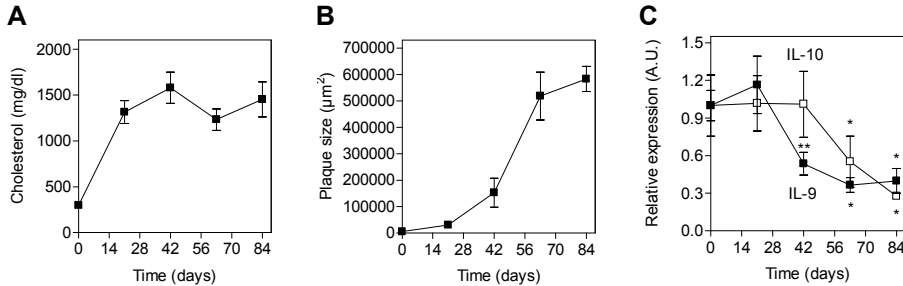


Fig. 3: Atherosclerosis, plasma cholesterol and endogenous splenic IL-9 expression. LDLr deficient mice were fed a Western-type diet for 84 days. At several time points after the start of the diet mice were killed and the total cholesterol levels in plasma were determined (**A**), the atherosclerotic lesions in the aortic root were quantified (**B**), and RNA was isolated from the spleen to quantitatively monitor the expression of IL-9 and IL-10 (**C**). Data are expressed as mean±SEM (n=6-9).

IL-9 and foam cell formation

Recently, IL-9 was shown to directly affect human macrophage metabolism³⁰ and we determined the effect of IL-9 on macrophage metabolism during atherosclerosis and focused on the first hallmark of atherosclerosis: foam cell formation. Isolated peritoneal macrophages from LDLr deficient mice rapidly transformed into foam cells by incubation with β -VLDL (**Fig. 4A**). The addition of IL-9 significantly decreased the amount of lipid stored by peritoneal macrophages by 55.5%, indicating that IL-9 directly affects lipid metabolism in addition to inflammatory responses (**Fig. 4B/C**).

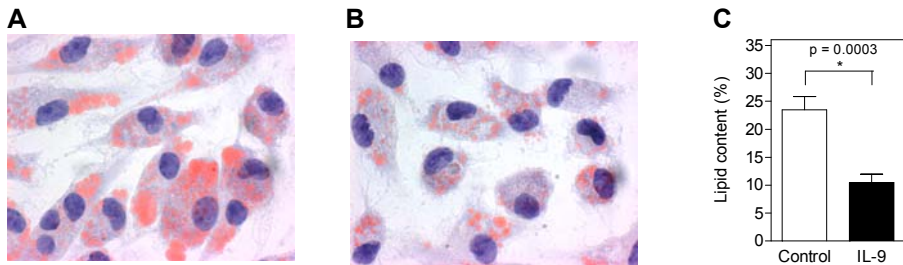


Fig. 4: Effect of IL-9 on β -VLDL induced foam cell formation. Isolated mouse peritoneal macrophages were incubated for 24 hours with or without β -VLDL (100 μ g/ml) in the absence (**A**) or presence (**B**) of IL-9 (40 ng/ml). Lipids were subsequently stained using Oil Red O (**A,B**) and the stained area was quantified (**C**). Data are expressed as mean±SEM (n = 13).

IL-9 and endothelial cell activation

Next, we determined the effect of IL-9 on endothelial cells and focused at the first step in atherosclerosis: adhesion of monocytes to endothelial cells. The mouse endothelial cell line H5V was activated by LPS in the presence or absence of IL-9 and subsequently the adherence of untreated RAW264.7 cells was determined. Incubation with IL-9 did not affect the number of macrophages adhering to the endothelial cells (not shown), whereas LPS incubation significantly elevated the number of macrophages that adhered to the endothelial cells (**Fig. 5**). Incubation of the endothelial cells with IL-9 plus LPS reduced the number of the macrophages that adhered to the endothelial cells with 20.9% as compared to the endothelial cells that were treated with LPS ($p=0.032$). Analysis of the expression of several adhesion molecules in endothelial cells in the various situations revealed that LPS induced the expression of VCAM-1, ICAM-1 and MCP-1, whereas IL-9 was able to reduce the induction of these molecules by LPS (data not shown).

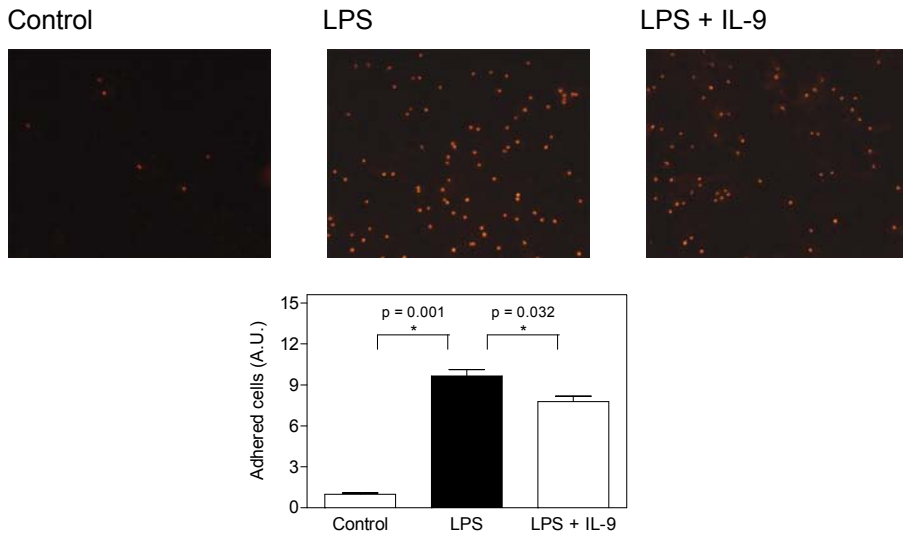


Fig. 5: Effect of IL-9 on monocyte adhesion to endothelial cells. H5V endothelial cells were preincubated with IL-9 (40 ng/ml) and subsequently activated with LPS (50 ng/ml) in the presence or absence of IL-9 (40 ng/ml). The endothelial cells were incubated with fluorescently labeled RAW cells and the number of adherent RAW cells per field was determined. Data are mean (arbitrary units) \pm SEM of three experiments.

DISCUSSION

The present manuscript underlines that one of the major targets for the development of new therapies for atherosclerosis will be the management of the inflammatory response, which is one of the hallmarks both in development and in progression of atherosclerosis¹⁻³. Reduction in the amount of pro-inflammatory mediators may be achieved by enhancing the anti-inflammatory response and this may be considered one of the main targets for therapy development. IL-9 is one of the typical Th2 cytokines and it has been associated with the development of asthma, but its relation to

atherosclerosis and more in general autoimmune-like inflammatory responses is less well studied.

In the present paper we show that the blockade of endogenous IL-9 using a vaccination approach³⁴ aggravated the extent of atherosclerosis in LDLr deficient mice by 200%. This implies that endogenously produced IL-9 protects LDLr deficient mice to a yet unknown and large extent from the development of atherosclerosis. The mechanism behind the protective activity of IL-9 on atherosclerosis may be deduced from the observation that IL-9 expression increases after the recovery from a sublethal bacterial infection with *P. aeruginosa*³⁰. During the recovery from the bacterial infection, IL-9 is upregulated and induces an anti-inflammatory response: IL-9 reduces the production of pro-inflammatory cytokines such as TNF- α , IL-12 and IFN- γ , while it induces the expression of the anti-inflammatory cytokine IL-10. These effects of IL-9 may also explain the beneficial effects of IL-9 in LDLr deficient mice on a Western-type diet, since we recently showed that blockade of endogenous IL-12 reduces atherosclerosis and one of the major effects of the blockade of IL-12 was the reduction in IFN- γ expression in the atherosclerotic plaque⁴⁸. In addition IL-9 may induce TGF- β production in human monocytes, which accounts for the capacity of IL-9 to reduce the TNF- α response of these cells to LPS³⁰. Mallat *et al.* have shown that the inhibition of the activity of TGF- β in apoE deficient mice favors the development of lesions with increased inflammatory component and decreased collagen content^{53,54}. One may therefore speculate that induction of TGF- β by IL-9 in monocytes and macrophages can also be partly responsible for the protective effects of IL-9 on the initiation of atherosclerosis in our experiments.

Our data indicate that the expression of IL-9 is apparently too low to prevent the initiation of atherosclerosis in control treated LDLr deficient mice, which may relate to the fact that the LDLr deficient mice in this study were predominantly on a C57Bl/6 background (>90%) and the C57Bl/6 background has previously been found to be associated with a strongly diminished production of IL-9, most probably as a consequence of a genetic defect⁴⁷. Specific strains of mice with high levels of endogenous IL-9, such as the DBA2 strain, are prone to develop asthma in contrast to C57Bl/6 mice, which have low levels of IL-9⁴⁷. The susceptibility of DBA2 mice to develop asthma is inversely related to their susceptibility to develop atherosclerosis⁵⁵. Interestingly, the expression of IL-9 in the major immune organ, the spleen, is even further reduced by feeding a Western-type diet to the LDLr deficient mice and the decrease in IL-9 expression even preceded the development of atherosclerotic plaques in the aortic root. It is therefore tempting to speculate that the endogenous levels of IL-9 are related to the occurrence of atherosclerosis.

The role of endogenous IL-9 in the development of atherosclerosis in mice is in line with the role of endogenous IL-10, which was shown to be protective against atherosclerosis in studies using transgenic mice. The effect of IL-10 was explained by its inhibitory effect on the expression of pro-inflammatory cytokines, monocyte infiltration, and reduction in expression of adhesion molecules by endothelial cells¹⁸⁻²⁹. As indicated, IL-9 shares at least some of the features of IL-10, since IL-9 is able to reduce the

expression of pro-inflammatory cytokines during an inflammatory stimulus and diminish the activation of monocytes^{30,31}.

In addition to the data on the role of endogenous IL-9, we also demonstrate that recombinant IL-9 (rIL-9) may be an interesting therapeutic approach to treat atherosclerosis. Our data show that both in male and female LDLr deficient mice rIL-9 inhibits the extent of atherosclerosis by approximately 60%. No effects of rIL-9 treatment were observed with respect to the plasma cholesterol levels, which is in contrast to our data on the effect of Ad.IL-10 on plasma cholesterol levels²⁹. Apparently, the effect of IL-9 on IL-10 expression is not strong enough to induce a reduction in plasma cholesterol levels. The effect of IL-9 on atherosclerosis is in part explained by the capacity of IL-9 to reduce the expression of IL-12 and IFN- γ and the induction of IL-10. The extent of inhibition of atherosclerosis by rIL-9 is comparable to the capacity of IL-10 to reduce atherosclerosis in the same animal model. In addition to these more general effects of IL-9 on the immune response, we determined that IL-9 affects two processes that are directly linked to the formation of the atherosclerotic plaque: monocyte adhesion and foam cell formation. Monocyte adhesion to LPS activated endothelial cells was to a limited extent (20%) inhibited by IL-9, while the foam cell formation in peritoneal macrophages was more than 2-fold reduced by IL-9. The reduction in both processes upon IL-9 treatment evidently contributes to the anti-atherosclerotic effect of IL-9 and may also explain that the plaques are reduced upon IL-9 treatment but the plaque composition is not affected. In order to fully determine the therapeutic potential of IL-9, it will be necessary to determine the effect of IL-9 on advanced, pre-existing plaques. IL-9 is a rather weak growth factor for mast cells, which have been implicated in some studies in the maintenance of the inflammatory process in more advanced plaques⁵⁶. Future studies will have to elucidate whether this will complicate the use of IL-9 as a drug for the treatment of atherosclerosis in man.

The use of IL-9 to treat atherosclerosis may be conflicting with the facilitating effect of IL-9 on the occurrence of asthma. It should, however, be noted that IL-9 is not a prerequisite for the development of asthma and asthma may develop in the absence of IL-9⁵⁷.

We conclude that endogenous IL-9 plays a modulating role in the process of atherosclerosis and high levels of endogenous IL-9 may be related to a lower incidence of atherosclerosis. In addition, IL-9 may be used as a drug to downregulate the inflammatory response during the formation and progression of the atherosclerotic plaque and thereby reduce the occurrence of cardiovascular disease.

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