**Vaccination against atherosclerosis**
Es, T. van

**Citation**
Es, T. van. (2009, January 29). *Vaccination against atherosclerosis*. Retrieved from https://hdl.handle.net/1887/13450

| Version: | Corrected Publisher's Version |
| --- | --- |
| License: | Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden |
| Downloaded from: | https://hdl.handle.net/1887/13450 |

**Note:** To cite this publication please use the final published version (if applicable).

# Chapter 4

## Attenuated atherosclerosis upon interleukin-17 receptor signaling disruption in LDL receptor deficient mice

Thomas van Es, Gijs H.M. van Puijvelde, Filip M.E. Segers, Ingrid M. Michon, Paula de Vos, Theo J.C. van Berkel and Johan Kuiper

*Submitted for publication*

## ABSTRACT

Atherosclerosis is an inflammatory disease, which is illustrated by the influx of macrophages and T cells in the sub-endothelial layer. IL-17 is an important cytokine, which bridges the innate and adaptive immune response and moreover IL-17 is involved in the transition from an acute into a chronic inflammation.

In this study we investigated the role of IL-17 receptor signaling in atherosclerosis. We therefore performed a bone marrow transplantation with IL-17 receptor deficient donor bone marrow into LDL receptor deficient recipient mice. After full bone marrow reconstitution a Western-type diet feeding was started and atherosclerotic lesions were quantified after 12 weeks.

A 46% reduction in lesion size in the aortic root was observed ($P<0.05$). Furthermore, a decrease in auto-antibodies against oxLDL was detected. The inflammatory status of IL-17 deficient bone marrow recipients was changed as indicated by the reduced IL-6 production by the spleen and increased IL-10 production within the HLN and PBMCs.

In conclusion, the IL-17 signaling is involved in the aggravation of atherosclerosis. This is probably mediated by a decrease in IgG anti-oxLDL antibodies and a change in the inflammatory status. Therefore, interfering in the IL-17 receptor pathway could provide a new therapeutic approach for inhibiting atherosclerosis lesion development.

## INTRODUCTION

Atherosclerosis is an inflammatory disease, which involves both components of the immune system, the adaptive and innate immune system[1]. The inflammatory response is tightly regulated by several mechanisms for example via interleukins (IL).

IL-17 is involved in the early activation of the immune system and plays an important role in bridging the innate immune response with the adaptive immune response.[2] IL-17 is mainly produced by T cells, especially $CD4^+$ effector memory T cells and has more recently been linked to a new class of T helper cells, the Th17 cells.[3, 4] IL-17 has a protective role, since IL-17 protects against infectious microorganisms such as *Klebsiella pneumoniae* , *Candida albicans* and *Toxoplasma gondii*.[5,6] On the other hand an elevated concentration of IL-17 is associated with different autoimmune diseases such as, rheumatoid arthritis and multiple sclerosis, where IL-17 plays a pathogenic role. [7, 8]



The IL-17 family comprises six members (IL-17A, B, C, D, E and F) and the best characterized member is IL-17A, which is also designated as IL-17 as it is the founding member of the IL-17 family. The receptor for IL-17 (IL-17R) is a type I transmembrane protein consisting of a 293 amino acids long extracellular domain and a relatively long intra cellular domain consisting of 525 amino acids.[9, 10] The IL-17 receptor is widely expressed with a prominent mRNA expression in lung, kidney, liver, spleen and also in isolated fibroblasts, endothelial cells, mesothelial cells and myeloid cells from mice.[9] This wide expression is also seen in humans, were the IL-17R for example is found on peripheral blood T cells and vascular endothelial cells. [11,12] The pathogenic role of IL-17 in autoimmunity and the parallel in function with proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and IL-1β makes IL-17 an interesting target for studying its role in atherosclerosis.[13]

IL-17 induces the expression of a wide range of proinflammatory cytokines and chemokines in various cell types as a consequence of the broad expression of IL-17R.[14] IL-17 stimulates the expression of IL-6 and CXCL8 (IL-8) by stromal cells and ICAM-1 by fibroblasts and keratinocytes.[9, 10, 15] Even more interesting is the effect of IL-17 on macrophages, which produce IL-1β, IL-1Ra, IL-6, IL-10, TNF-α and prostaglandin $E_2$ ($PGE_2$) in response to IL-17. Matrix metalloproteinase (MMP)-3 and MMP-9 are also induced by IL-17.[16, 17] These proteinases and proinflammatory interleukins and chemokines have already been implicated in atherosclerotic lesion growth and destabilization of the plaque.[18-20]

The role of the IL-17R signaling pathway, although extensively studied in other autoimmune diseases, is not yet established in the process of atherosclerosis. Therefore we transplanted bone marrow of $IL\text{-}17R^{-/-}$ mice to $LDLr^{-/-}$ mice, which

resulted in a 46% reduction in atherosclerotic lesion size. These data indicate an aggravating role of IL-17 in the process of atherosclerosis and establish a new target to beneficially influence atherosclerosis lesion development.

## METHODS

### ANIMALS

All animal work was approved by Leiden University and was in compliance with the Dutch government guidelines. LDL receptor deficient ($LDLr^{-/-}$) mice were purchased from Jackson Laboratories. IL-17 receptor knockout mice were a kind gift from J. Peschon (Amgen, Seattle, WA) and created as described by Ye *et al.*[6] The mice were kept under standard laboratory conditions and food and water were provided *ad libitum*.

### BONE MARROW TRANSPLANTATION (BMT) AND INDUCTION OF HYPER-CHOLESTEROLEMIA

To induce bone marrow aplasia, male $LDLr^{-/-}$ mice were exposed to a single dose of 9 Gy (0.19 Gy/min, 200 kV, 4 mA) total body irradiation, using an Andrex Smart 225 Röntgen source (YXLON Int, Copenhagen, Denmark) with a 6-mm aluminum filter. Bone marrow was isolated by flushing the femurs and tibias from mice with phosphate-buffered saline (PBS). Single-cell suspensions were prepared by passing the cells through a 30 µm nylon gauze. Irradiated recipients received $0.5x10^7$ bone marrow cells by intravenous injection into the tail vein. After a recovery of 8 weeks animals received a Western-type diet *ad libitum* containing 15% cocoa butter and 0.25% cholesterol (Special Diet Services, Witham, Essex, UK) for 12 weeks. During the experiment the mice were weighted every week and checked for well-being.

### SERUM LIPID LEVELS

Every 3 weeks the serum cholesterol levels were determined to assess the effect of the Western-type diet. Blood samples were collected by tail bleeding from non-fasting animals. Total cholesterol levels were quantified spectrophotometrically using an enzymatic procedure (Roche Diagnostics, Germany). Precipath standardized serum (Boehringer, Germany) was used as an internal standard.

### HISTOLOGY AND IMMUNOHISTOCHEMISTRY

Mice were anaesthetized with ketamine-hypnorm and perfused with PBS and subsequently with FormalFixx. The heart and complete aorta were removed. The heart was embedded in OCT compound (TissueTek; Sakura Finetek, The Netherlands) and cryosections of 10 µm were made of the aortic root containing the aortic valves. Cryosections were routinely stained with Oil-Red-O and hematoxylin (Sigma Diagnostics, MO). Corresponding sections on separate slides were stained immunohistochemically for macrophages using an antibody against a macrophage-specific antigen (MoMa-2, Research Diagnostics Inc.) and for collagen using Masson trichrome staining according to manufacturer's protocol (Sigma Diagnostics). Neutrophils were stained by specific esterase staining (Naphthol AS-D chloroacetate, Sigma). Mast cells were stained by Toluidin Blue Staining (Sigma). The different histological stains were quantified using a Leica DM-RE microscope and Leica Qwin Imaging software (Leica Ltd., Germany).



### FACS ANALYSIS OF LEUKOCYTES

Peripheral Blood Mononuclear Cells (PBMC) were isolated via orbital bleeding and erythrocytes were removed by incubating the cells with erythrocyte lysis buffer (0.15 M $NH_4Cl$, 10 mN $NaHCO_3$, 0.1 mM EDTA, pH 7.3). Spleens, Heart lymph nodes (HLN) and Mesenteric lymph nodes (MNL) were dissected from the mice and single cell suspension was obtained by passing the organs through a 70 µm cell strainer (Falcon, The Netherlands). Cells were stained with surface markers (0.20 µg antibody/300.000 cells) and subsequently analyzed by flow cytometric analysis. The F4/80-FITC and CD19-FITC antibody were used for the detection of macrophages and B cells, respectively (Immunosource, Belgium). The unlabeled antibody for IL-17R was purchased from R & D systems and as a secondary antibody anti goat-IgG-PE (Abcam, UK) was used according manufacturers protocol. All data were acquired on a FACScalibur and analyzed with CELLQuest software (BD Biosciences, The Netherlands).

### OXLDL ANTIBODY DETECTION

Cu-oxLDL was synthesized as described previously [21, 22]. Antibodies against Cu-oxLDL were determined according to Damoiseaux et al.[23] MaxiSorp 96 wells plates (Nunc, Roskilde, Denmark) were coated overnight with 100 µg oxLDL in coating buffer (50mM $NaHCO_3$, 50mM $Na_2CO_3$, pH=9.6) at 4 $^0$C. IgM, IgG2a and IgG1 antibodies directed against oxLDL were detected with an isotype Ig detection kit according manufacturer’s protocol (Zymed lab. Inc., CA).

### Cytokine production

Peripheral Blood Mononuclear Cells (PBMC), spleens, heart lymph nodes (HLN) and mesenteric lymph nodes (MNL) were dissected and a single cell suspension was made as described above. Subsequently the cells were cultured in a 96-wells round bottom plate which was coated with α-CD28 and α-CD3 (0.25 μg/well) at a cell density of $2.10^5$ cells per well. Cells were cultured in RPMI 1640 (with L-Glutamine) supplemented with 10 % FCS, 100 U/ml penicillin and 100 μg/ml streptomycin (all from BioWhittaker Europe). Supernatant was used for IL-10 and IL-6 ELISA's according to manufacturer's protocol (both from eBioscience, Belgium).

### Statistical analysis

All data are expressed as mean ± SEM. The two-tailed student's t-test was used to compare individual groups of mice or cells. When indicated, a Mann-Whitney test was used to analyze not normally distributed data. *P* values of <0.05 were considered significant.

## RESULTS

### Efficacy of IL-17R$^{-/-}$ bone marrow transplantation

To asses the efficacy of IL-17R$^{-/-}$ BMT, we determined the IL-17R expression by FACS analysis. 20 weeks after BMT the IL-17R expressing cells in different organs were analyzed. The number of IL-17R expressing cells was significantly reduced with 85% within the blood (Figure 4.1A, *P*<0.01). Furthermore, the IL-17R expression on circulating macrophages was analyzed. We observed a strong reduction of 84% in IL-17R expressing macrophages in IL-17R$^{-/-}$ transplanted mice (Figure 4.1B). Within the spleen of IL-17R$^{-/-}$ transplanted mice the expression of IL-17R on mRNA level was also stongely reduced (Figure 4.1C). These data demonstrate an effective replacement of acceptor bone marrow by IL-17R$^{-/-}$ donor bone marrow.

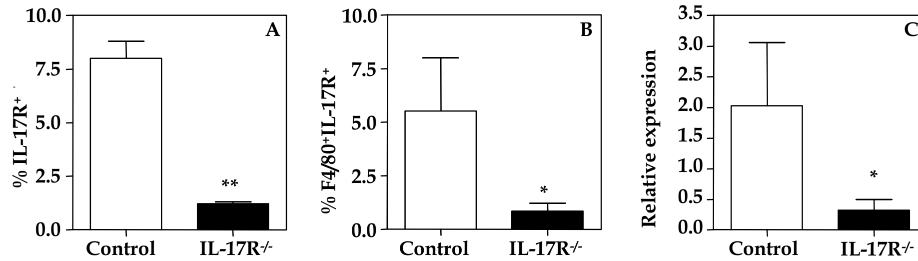


**FIGURE 4.1: IL-17 RECEPTOR EXPRESSING CELLS OF PBMCS IN IL17R-/- BM RECIPIENT AND CONTROL MICE.** After sacrificing the mice, PBMCs were stained with an antibody directed against IL-17R and F4/80 or IL-17R alone and analyzed with a FACS machine. The expression of the IL-17R was significant lower within IL-17R$^{-/-}$ transplanted mice (A). Within the macrophage population of the PBMCs IL-17R was also significant lower in IL-17R$^{-/-}$ transplanted mice (B). Total RNA was isolated from the spleen cells and the expression of the IL-17R was assessed by qPCR analysis, illustrating almost complete abolishment of IL-17R expression IL-17R$^{-/-}$ transplanted mice (C). Control mice (N=5) and IL-17R$^{-/-}$ BM recipients (N=5). *$P$< 0.05, **$P$<0.01



## EFFECT OF IL-17R$^{-/-}$ BMT IN ATHEROSCLEROSIS

Next we determined the effect on IL-17R$^{-/-}$ BMT in atherosclerosis whereby the mice were fed a Western-type diet for 12 weeks. The mice were subsequently sacrificed and the aorta and the aortic root were analyzed for the atherosclerotic burden. Atherosclerotic lesions were quantified in the aortic root of IL-17R$^{-/-}$ BM recipients (Figure 4.2B) and control transplanted mice (Figure 4.2A). In the IL-17R$^{-/-}$ BM recipients a significant reduction of 46% in plaque size was observed compared to the control group (Figure 4.2C; 245,000 ± 43,700 versus 454,000 ± 91,200; $P$<0.05).

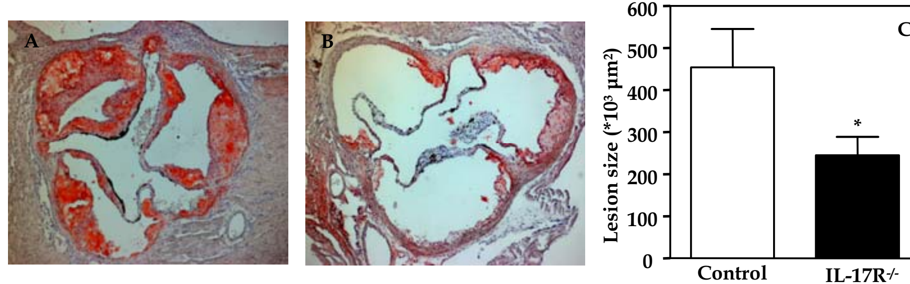


**FIGURE 4.2: IL-17R$^{-/-}$ BM RECIPIENTS DEMONSTRATE REDUCED LESION SIZE AT THE AORTIC ROOT.** After BMT the mice were fed a Western-type diet for 12 weeks and were sacrificed. Cryosections of the aortic root of the control group (A) and the IL-17R$^{-/-}$ BM recipients (B) were made and subsequently stained for lipid with Oil-red-O. Within the IL-17R$^{-/-}$ BM recipients (open bar, N=10) there is a reduction of 46% in lesion size when compared to the control mice (closed bar, N=8) (C). *$P$<0.05

### EFFECT OF IL-17R DEFICIENCY ON CHOLESTEROL LEVELS

After the BMT we observed no difference in bodyweight between the IL-17R$^{-/-}$ BM recipients and control group (Figure 4.4A). The drop in weight during the first week is characteristic for bone marrow transplantation. In week 9 both groups of mice were put on a Western-type diet (0.25% cholesterol) to initiate atherosclerosis. During Western-type diet feeding serum cholesterol levels were determined and no significant difference was observed between mice which received IL-17R$^{-/-}$ and wild-type bone marrow (Figure 4.4B).

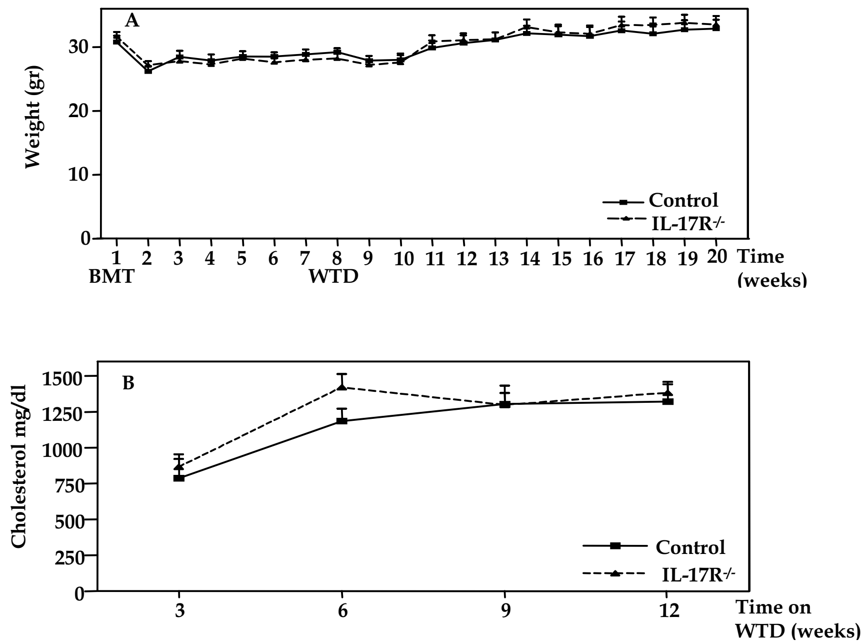


**FIGURE 4.4: BODYWEIGHT AND CHOLESTEROL LEVELS IN IL-17R$^{-/-}$ AND CONTROL BMT MICE.** During the experiment the animal were weighted to study well being (A). The mice were fed a Western-type diet and every three weeks blood samples were taken and cholesterol levels in the serum were determined (B).

### PLAQUE COMPOSITION IS ALTERED AFTER IL-17R$^{-/-}$ BMT

IL-17R signaling is involved in the regulation of different MMP's[24] and recruitment of polymorphonuclear leukocytes.[25] Therefore we assessed whether a bone marrow transplantation with IL-17R$^{-/-}$ BM affected morphological parameters and composition within the lesions in the aortic root. To assess the collagen content and thus stability of the plaque, a Masson trichrome staining was performed. The collagen content within the lesion was not altered in IL-17R$^{-/-}$ BM recipient mice compared to control mice, indicating that the plaque stability is not affected

(Figure 4.5A; 0.12 ± 0.04 versus 0.14 ± 0.03, *P*=0.63). To examine the number of macrophages in the lesion, a MoMa-2 staining was performed. Interestingly, we observed a 25 % increase in macrophage content within the plaque of mice which received IL-17R$^{-/-}$ BM compared to control mice (Figure 4.5B; 0.32 ± 0.02 versus 0.43 ± 0.03, *P*=0.01). There was no significant difference in neutrophils content between the control group and the IL-17R$^{-/-}$ BM recipients (Figure 4.5C; 2.14 ± 0.63 versus 1.14 ± 0.36, *P*=0.29). Interestingly, IL-17R$^{-/-}$ BM recipients demonstrated a 43.8% reduction in the number of mast cells as determined by toluidin blue staining in the aortic root section when compared to controls (Figure 4.5D; 4.18 ± 0.96 versus 7.45 ± 1.20, *P*<0.05)

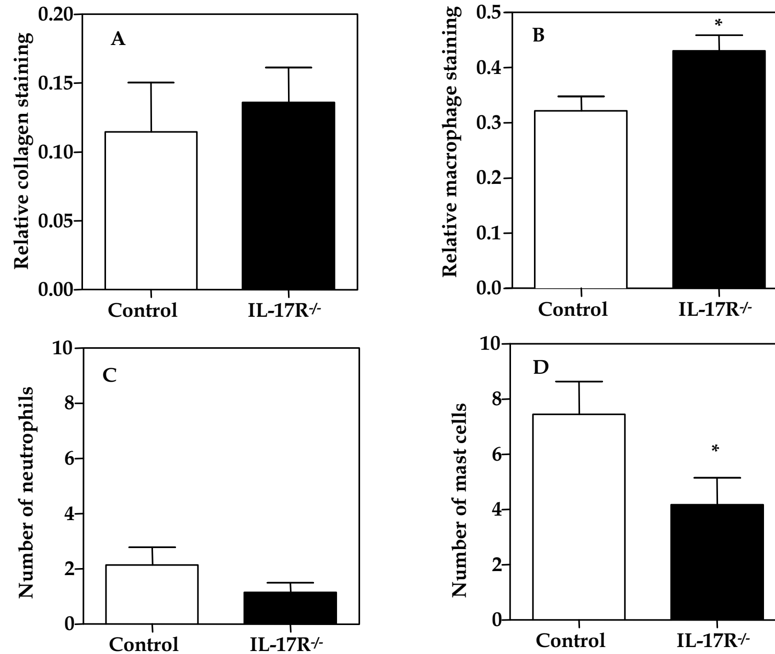


**FIGURE 4.5: COMPOSITION OF PLAQUE IN THE AORTIC ROOT IN IL-17R$^{-/-}$ BM RECIPIENT AND CONTROL MICE.** Cryosections of the aortic root of control mice (open bars) and IL-17R$^{-/-}$ BM recipients (closed bars) were stained. There is no difference in collagen content within the plaque (A). The relative macrophage within the intima is significantly increased in the IL-17R$^{-/-}$ BM recipients (B). The amount of neutrophils within the plaque does not change in the IL-17R$^{-/-}$ BM recipient mice (C). Mast cells were significantly reduced in the IL-17R$^{-/-}$ BM recipient mice (D). *$P<0.05$

### REDUCTION OF IL-17R$^{+}$ B CELLS AND REDUCED LEVELS OF AUTOANTIBODIES IN IL-17R$^{-/-}$ BM RECIPIENTS

Recently, the role of IL-17R expressing B cells is described in relation to germinal center (GC) activity and spontaneous development of antibody mediated autoimmunity.[26] As the IL-17R$^{-/-}$ BM recipients have reduced IL-17R expressing B

cells in the lymphoid organs (Figure 4.6A-C), we studied the effect thereof on the formation of oxLDL specific autoantibodies. We observed a significant reduction of 33% in IgG antibodies directed against oxLDL in serum of mice that had received bone marrow from IL-17R deficient donors (Figure 4.6B, *P*<0.05).

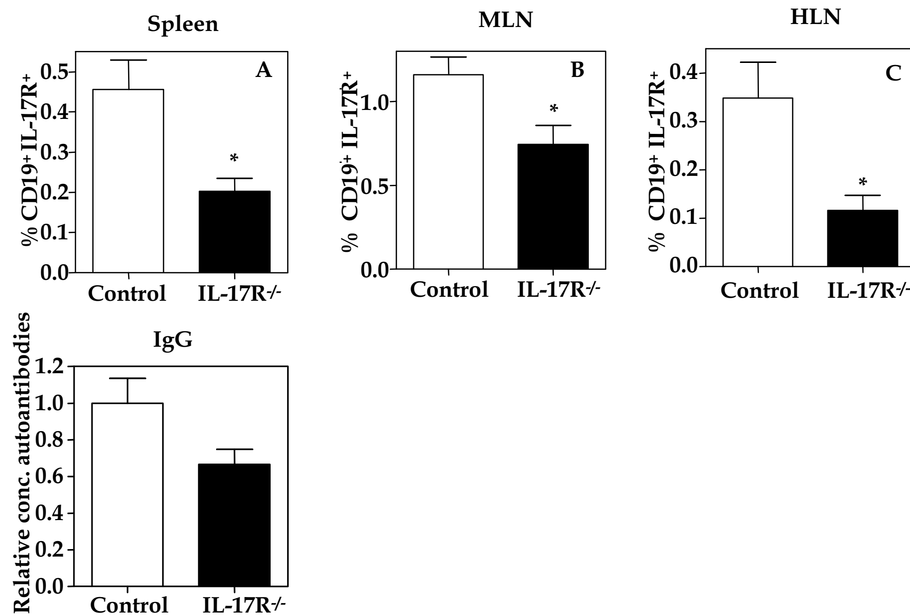


**FIGURE 4.6: REDUCED IL-17R+ B CELLS AND REDUCED AUTOANTIBODIES AGAINST OXLDL IN IL-17R-/- BM RECIPIENT MICE.** Single cell suspensions of lymphoid organs of control mice (white bars) and IL-17R-/- BM recipient mice (black bars ) was obtained and stained for CD19 and IL17R and subsequent analyzed with a FACS machine. Within the spleen (A), MLN (B) and HLN (C) there is a significant decrease in IL-17R expressing B cells. The serum of control mice (open bars) and IL-17R-/- BM recipient mice (closed bars) was used for detection of antibodies directed against oxLDL (D). The level of total IgG autoantigens was significant lower in IL-17R-/- BM recipient mice. **P*<0.05

### DOWNSTREAM SIGNALING OF IL-17 IS IMPAIRED IN IL-17R-/- BM

IL-17 is involved in the activation of the immune system and therefore we wanted to study the effect of IL-17R deficiency on the production of interleukins by lymphoid cells. First we studied IL-6, a prominent downstream effector product of IL-17 signaling.[9] We assessed IL-6 production with an ELISA on supernatant of α-CD28 and α-CD3 activated cells from several lymphoid organs. The IL-6 production dropped 66% in the spleen of IL-17R-/- BM recipients compared to control (Figure 4.7A; 215.17 ± 114.60 versus 635.5 ± 148.22 pg/ml, *P*<0.05), whereas there was no significant change detected in the MLN, HLN and PBMCs.

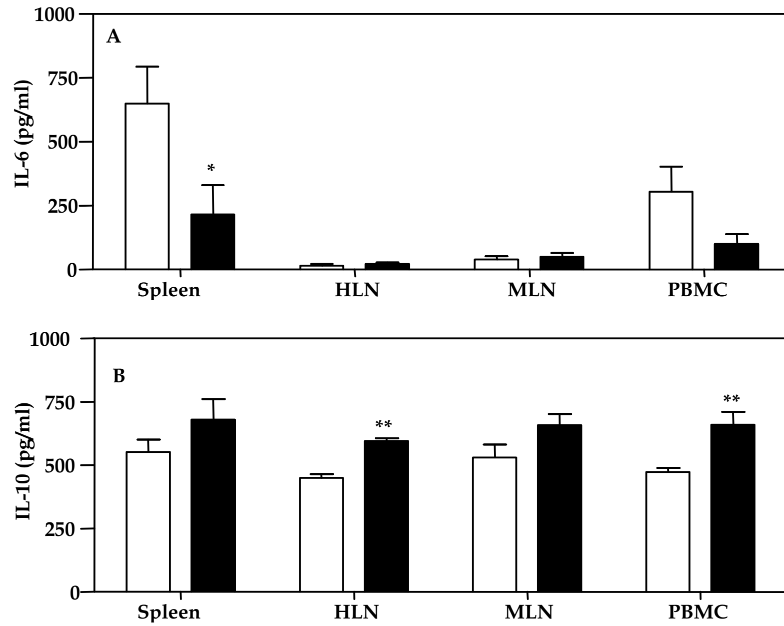




**Figure 4.7: Cytokine profile is changed in IL-17R-/- recipient mice.** The spleen, HLN, MLN and PBMCs of control mice (open bars) and IL-17R-/- BM recipient mice (closed bars) were removed. *Ex vivo* stimulated lymphoid cells were used to analyze the cytokine production with an ELISA. The IL-6 expression is decreased in spleen cells of IL-17R-/- recipient mice (A). IL-10 was significant induced in HLN and PBMCs of IL-17R-/- recipient mice(B) *$P$<0.05, **$P$<0.01

Furthermore, we determined IL-10 production with an ELISA on activated lymphoid cells from different lymphoid organs. Interestingly, we observed a very significant increase in IL-10 expression of 24.61% in HLN and an increase of 28.28% in PBMCs in IL-17R-/- BM recipients (Figure 4.7B: HLN, 449.33 ± 15.20 versus 596.00 ± 10.54, $P$<0.01; PBMCs, 473.33 ± 16.14 versus 660.00 ± 50.71, $P$<0.01). However, within the spleen and MLN IL-10 production was not changed.

## DISCUSSION

The receptor for IL-17 (IL-17R) is a type I transmembrane protein that is ubiquitously expressed in the body.[9] IL-17 exhibits pleiotropic biological actions on various atheroma-associated cell types, such as endothelial cells, vascular smooth muscle cells and macrophages.[16, 17, 24] Upon activation by IL-17 these cells produce pro-inflammatory cytokines, chemokines and matrix metalloproteinases (MMPs), including TNF-α, IL-1β, IL-6, CXCL8, CCL2 and MMP-9.[18-20, 24] Although, IL-17 is

considered to be an important interleukin in several autoimmune diseases, research on the role of IL-17 in atherosclerosis is limited.

To investigate the role of IL-17 signaling in atherosclerosis, we performed a BMT with IL-17R$^{-/-}$ donor bone marrow into LDLr$^{-/-}$ recipients and evaluated the effect thereof on atherosclerosis after 12 weeks of Western-type diet. First, we verified whether the BMT was successful in replacing BM cells. We therefore analyzed the expression of IL-17R in the spleen with qPCR and observed a large reduction in IL-17R expression in IL-17R$^{-/-}$ transplanted mice. Furthermore, we analysed IL-17R expression with FACS analysis in PBMCs. We observed a large reduction of 85% in IL-17R expressing cells in the PBMCs, indicating that the BMT with IL-17R$^{-/-}$ bone marrow was successful and cells were effectively replaced by donor IL-17R$^{-/-}$ bone marrow. The reduction in IL-17R was further specified to macrophages, were we observed a reduction of 84% in the expression of the IL-17R. This finding is in line with previous bone marrow transplantation experiments in our laboratory, for example with CCR2 deficient bone marrow. [27]

Next, we studied the effect of IL-17R deficiency on atherosclerosis. We observed a striking reduction in plaque size within the aortic valve region. This effect was independent of cholesterol levels and bodyweight as these parameters were unchanged between the IL-17R$^{-/-}$ transplanted and control transplanted mice. IL-17 signaling is involved in the regulation of different MMPs.[24] Therefore we studied plaque stability, since MMPs, such as MMP-9, are well-known for their plaque destabilizing potential.[19] We did however not detect any changes in collagen content, a marker for plaque stability, within the lesion. It should be noted that MMPs are also produced by smooth muscle cells and these cells are not (effectively) replaced by IL-17R$^{-/-}$ deficient bone marrow cells. Therefore, smooth muscle cells still expres the IL-17R and are thus responsive to IL-17 within the lesion.

Interestingly, we observed an increase in relative macrophage content within the plaque of IL-17R$^{-/-}$ transplanted mice, which is in agreement with the decreased lesion size, as more initial plaques show a higher proportion of macrophages.[28] The macrophages within the plaque are likely to be IL-17R deficient and thus not, or to a lesser extent, responding to IL-17 within the plaque. This impaired IL-17 signaling pathway may lead to a diminished production of pro-inflammatory interleukins, chemokines and proteinases, which have been implicated in lesion growth and destabilization. Furthermore, we observed a significant reduction in mast cells within the lesion of the IL-17R$^{-/-}$ BM recipients. Mast cells are more prominent in advanced stages of atherosclerotic plaque development, so their reduced presence do agree with the less advanced stage of lesion formation in the IL-17R$^{-/-}$ BM recipients.[29,30, 31] IL-17 is able to induce the production of eotoxin-1 (CCL11), which is an important chemoattractant for mast

cells and is detected in atherosclerotic lesions.[32, 33] IL-17R$^{-/-}$ BM recipients have an impaired IL-17 signaling and thus the eotoxin signaling may also be impaired, leading to the decreased number of mast cells within the atherosclerotic plaque in the IL-17R$^{-/-}$ transplanted mice. In contrast to the mast cells, we did not observe any change in neutrophil count within the plaque of IL-17R$^{-/-}$ BM recipients. This is surprising as IL-17 is involved in CXCL-1 mediated neutrophil recruitment.[34] However, neutrophils are normally observed in very low numbers within the atherosclerotic lesion in the aortic valve.

Impairment in IL-17 signaling may also affect the general inflammatory status, since IL-17 is involved in bridging the innate and adaptive immune response.[2, 35] We determined the expression of IL-6 and IL-10. IL-6 is a proinflammatory cytokine that provokes a broad range of cellular and physiological responses and was one of the earliest defined IL-17 induced target genes.[3, 9] Indeed, within the spleens of IL-17R$^{-/-}$ BM recipients we observed a lower expression of IL-6. Interestingly, IL-10 expression is significantly increased in IL-17R$^{-/-}$ BM recipients. IL-10 is known to reduce atherosclerosis in LDLr$^{-/-}$ mice upon overexpression.[36-38]



Recently, Hsu *et al* suggested that IL-17 may result in spontaneous generation of autoreactive GCs as IL-17 increases the retention of B cells within the GCs through modulation of the activity of the Regulators of G-protein signaling (RGS) genes.[26] In the spleens of mice transplanted with IL-17R$^{-/-}$ bone marrow, a reduction in IL-17R ecpressing B cells paralleled a reduction in anti-oxLDL IgG antibodies which is in line with the hypothesis of Hsu *et al*. Increased IgG autoantibodies against oxLDL are associated with a larger atherosclerotic burden, therefore this may provide an additional explanation for the reduced lesion size in the IL-17R$^{-/-}$ BM recipients.

The IL-17R signaling is an interesting target for clinical applications to modulate the immune response in atherosclerosis. IL-17 forms a bridge between the innate and adaptive immune response and plays a crucial role in the progression from acute to chronic inflammation.[2] In our laboratory we blocked IL-17 via DNA vaccination, which resulted in a decreased atherogenesis in LDLr$^{-/-}$ mice.[39] Furthermore, Th17 cells, profound producers of IL-17, have been identified in patients with acute coronary syndrome, underlining the potential role of IL-17 in atherosclerosis.[40]

In conclusion, we demonstrate that an impaired IL-17R signaling results in less atherosclerosis, indicating an aggravating role for IL-17 in this disease. Therapies interfering in the IL-17 pathway may provide a newly explored treatment against atherosclerosis.

## References

1. Hansson GK, Robertson AK, Soderberg-Naucler C. Inflammation and atherosclerosis. *Annu Rev Pathol.* 2006;1:297-329.
2. Yu JJ, Gaffen SL. Interleukin-17: a novel inflammatory cytokine that bridges innate and adaptive immunity. *Front Biosci.* 2008;13:170-177.
3. Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, Pin JJ, Garrone P, Garcia E, Saeland S, Blanchard D, Gaillard C, Das Mahapatra B, Rouvier E, Golstein P, Banchereau J, Lebecque S. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J Exp Med.* 1996;183:2593-2603.
4. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity.* 2006;24:677-688.
5. Kelly MN, Kolls JK, Happel K, Schwartzman JD, Schwarzenberger P, Combe C, Moretto M, Khan IA. Interleukin-17/interleukin-17 receptor-mediated signaling is important for generation of an optimal polymorphonuclear response against Toxoplasma gondii infection. *Infect Immun.* 2005;73:617-621.
6. Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P, Oliver P, Huang W, Zhang P, Zhang J, Shellito JE, Bagby GJ, Nelson S, Charrier K, Peschon JJ, Kolls JK. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *J Exp Med.* 2001;194:519-527.
7. Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, Saito S, Inoue K, Kamatani N, Gillespie MT, Martin TJ, Suda T. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J Clin Invest.* 1999;103:1345-1352.
8. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med.* 2005;201:233-240.
9. Yao Z, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, Cohen JI, Spriggs MK. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity.* 1995;3:811-821.
10. Yao Z, Painter SL, Fanslow WC, Ulrich D, Macduff BM, Spriggs MK, Armitage RJ. Human IL-17: a novel cytokine derived from T cells. *J Immunol.* 1995;155:5483-5486.
11. Silva WA, Jr., Covas DT, Panepucci RA, Proto-Siqueira R, Siufi JL, Zanette DL, Santos AR, Zago MA. The profile of gene expression of human marrow mesenchymal stem cells. *Stem Cells.* 2003;21:661-669.
12. Moseley TA, Haudenschild DR, Rose L, Reddi AH. Interleukin-17 family and IL-17 receptors. *Cytokine Growth Factor Rev.* 2003;14:155-174.
13. Kikly K, Liu L, Na S, Sedgwick JD. The IL-23/Th(17) axis: therapeutic targets for autoimmune inflammation. *Curr Opin Immunol.* 2006;18:670-675.
14. Kolls JK, Linden A. Interleukin-17 family members and inflammation. *Immunity.* 2004;21:467-476.
15. Kennedy J, Rossi DL, Zurawski SM, Vega F, Jr., Kastelein RA, Wagner JL, Hannum CH, Zlotnik A. Mouse IL-17: a cytokine preferentially expressed by alpha beta TCR + CD4-CD8-T cells. *J Interferon Cytokine Res.* 1996;16:611-617.
16. Jovanovic DV, Di Battista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, Mineau F, Pelletier JP. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. *J Immunol.* 1998;160:3513-3521.
17. Jovanovic DV, Di Battista JA, Martel-Pelletier J, Reboul P, He Y, Jolicoeur FC, Pelletier JP. Modulation of TIMP-1 synthesis by antiinflammatory cytokines and prostaglandin E2 in interleukin 17 stimulated human monocytes/macrophages. *J Rheumatol.* 2001;28:712-718.
18. Csiszar A, Ungvari Z. Synergistic effects of vascular IL-17 and TNFalpha may promote coronary artery disease. *Med Hypotheses.* 2004;63:696-698.
19. de Nooijer R, Verkleij CJ, von der Thusen JH, Jukema JW, van der Wall EE, van Berkel TJ, Baker AH, Biessen EA. Lesional overexpression of matrix metalloproteinase-9 promotes intraplaque hemorrhage in advanced lesions but not at earlier stages of atherogenesis. *Arterioscler Thromb Vasc Biol.* 2006;26:340-346.

20. Kirii H, Niwa T, Yamada Y, Wada H, Saito K, Iwakura Y, Asano M, Moriwaki H, Seishima M. Lack of interleukin-1beta decreases the severity of atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol.* 2003;23:656-660.
21. Redgrave TG, Roberts DC, West CE. Separation of plasma lipoproteins by density-gradient ultracentrifugation. *Anal Biochem.* 1975;65:42-49.
22. Van Berkel TJ, De Rijke YB, Kruijt JK. Different fate in vivo of oxidatively modified low density lipoprotein and acetylated low density lipoprotein in rats. Recognition by various scavenger receptors on Kupffer and endothelial liver cells. *J Biol Chem.* 1991;266:2282-2289.
23. Damoiseaux J, Jeyasekharan AD, Theunissen R, Tervaert JW. Cross-reactivity of IgM and IgG anticardiolipin antibodies with oxidized-low density lipoproteins. *Ann N Y Acad Sci.* 2005;1050:163-169.
24. Koenders MI, Kolls JK, Oppers-Walgreen B, van den Bersselaar L, Joosten LA, Schurr JR, Schwarzenberger P, van den Berg WB, Lubberts E. Interleukin-17 receptor deficiency results in impaired synovial expression of interleukin-1 and matrix metalloproteinases 3, 9, and 13 and prevents cartilage destruction during chronic reactivated streptococcal cell wall-induced arthritis. *Arthritis Rheum.* 2005;52:3239-3247.
25. Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol.* 2007;25:821-852.
26. Hsu HC, Yang P, Wang J, Wu Q, Myers R, Chen J, Yi J, Guentert T, Tousson A, Stanus AL, Le TV, Lorenz RG, Xu H, Kolls JK, Carter RH, Chaplin DD, Williams RW, Mountz JD. Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. *Nat Immunol.* 2008;9:166-175.
27. Guo J, de Waard V, Van Eck M, Hildebrand RB, van Wanrooij EJ, Kuiper J, Maeda N, Benson GM, Groot PH, Van Berkel TJ. Repopulation of apolipoprotein E knockout mice with CCR2-deficient bone marrow progenitor cells does not inhibit ongoing atherosclerotic lesion development. *Arterioscler Thromb Vasc Biol.* 2005;25:1014-1019.
28. Hansson GK, Jonasson L, Seifert PS, Stemme S. Immune mechanisms in atherosclerosis. *Arteriosclerosis.* 1989;9:567-578.
29. Kaartinen M, Penttila A, Kovanen PT. Accumulation of activated mast cells in the shoulder region of human coronary atheroma, the predilection site of atheromatous rupture. *Circulation.* 1994;90:1669-1678.
30. Bot I, de Jager SC, Zernecke A, Lindstedt KA, van Berkel TJ, Weber C, Biessen EA. Perivascular mast cells promote atherogenesis and induce plaque destabilization in apolipoprotein E-deficient mice. *Circulation.* 2007;115:2516-2525.
31. Lindstedt KA, Kovanen PT. Mast cells in vulnerable coronary plaques: potential mechanisms linking mast cell activation to plaque erosion and rupture. *Curr Opin Lipidol.* 2004;15:567-573.
32. Haley KJ, Lilly CM, Yang JH, Feng Y, Kennedy SP, Turi TG, Thompson JF, Sukhova GH, Libby P, Lee RT. Overexpression of eotaxin and the CCR3 receptor in human atherosclerosis: using genomic technology to identify a potential novel pathway of vascular inflammation. *Circulation.* 2000;102:2185-2189.
33. Rahman MS, Yamasaki A, Yang J, Shan L, Halayko AJ, Gounni AS. IL-17A induces eotaxin-1/CC chemokine ligand 11 expression in human airway smooth muscle cells: role of MAPK (Erk1/2, JNK, and p38) pathways. *J Immunol.* 2006;177:4064-4071.
34. Liang SC, Long AJ, Bennett F, Whitters MJ, Karim R, Collins M, Goldman SJ, Dunussi-Joannopoulos K, Williams CM, Wright JF, Fouser LA. An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment. *J Immunol.* 2007;179:7791-7799.
35. Ley K, Smith E, Stark MA. IL-17A-producing neutrophil-regulatory Tn lymphocytes. *Immunol Res.* 2006;34:229-242.
36. Von Der Thusen JH, Kuiper J, Fekkes ML, De Vos P, Van Berkel TJ, Biessen EA. Attenuation of atherogenesis by systemic and local adenovirus-mediated gene transfer of interleukin-10 in LDLr-/- mice. *Faseb J.* 2001;15:2730-2732.
37. Mallat Z, Besnard S, Duriez M, Deleuze V, Emmanuel F, Bureau MF, Soubrier F, Esposito B, Duez H, Fievet C, Staels B, Duverger N, Scherman D, Tedgui A. Protective role of interleukin-10 in atherosclerosis. *Circ Res.* 1999;85:e17-24.

38. Potteaux S, Esposito B, van Oostrom O, Brun V, Ardouin P, Groux H, Tedgui A, Mallat Z. Leukocyte-derived interleukin 10 is required for protection against atherosclerosis in low-density lipoprotein receptor knockout mice. *Arterioscler Thromb Vasc Biol.* 2004;24:1474-1478.
39. van Es T, Hauer AD, van Puijvelde GH, van Wanrooij EJ, de Vos P, Peterse N, van Berkel TJ, Kuiper J. Abstract 1353: DNA vaccination Against IL-17 Attenuates Atherosclerosis in LDL Receptor Deficient Mice. *Circulation*. 2006;114:II_257-b-.
40. Cheng X, Yu X, Ding YJ, Fu QQ, Xie JJ, Tang TT, Yao R, Chen Y, Liao YH. The Th17/Treg imbalance in patients with acute coronary syndrome. *Clin Immunol.* 2008;127:89-97.