Vaccine 29 (2011) 976–983

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

IL-15 aggravates atherosclerotic lesion development in LDL receptor deficient mice

Thomas van Es, Gijs H.M. van Puijvelde, Ingrid N. Michon, Eva J.A. van Wanrooij, Paula de Vos, Niels Peterse, Theo J.C. van Berkel, Johan Kuiper*

Division of Biopharmaceutics, LACDR, Leiden University, Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 15 April 2010
Received in revised form 5 November 2010
Accepted 15 November 2010
Available online 27 November 2010

Keywords:
Interleukin-15
Vaccination
Atherosclerosis
Inflammation
Mouse model

ABSTRACT

Background: Interleukin 15 (IL-15) is a pro-inflammatory cytokine involved in inflammatory diseases and IL-15 is expressed in atherosclerotic plaques.
Methods: To establish the role of IL-15 in atherosclerosis we studied the effect of IL-15 on atherosclerosis associated cells in vitro and in vivo by neutralizing IL-15 using a DNA vaccination strategy.
Results: Upon feeding a Western type diet LDLr$^{-/-}$ mice do express higher levels of IL-15 within the spleen and the number of IL-15 expressing cells among blood leukocytes and spleen cells is increased. Addition of IL-15 to macrophages induces the expression TNF-α and CCL-2. After the mice were vaccinated against IL-15, we observe a reduction in plaque size of 75% plaque. Unexpectedly, the relative number of macrophages within the plaque was 2-fold higher in IL-15 vaccinated mice than in control mice. Vaccination against IL-15 leads to an increased cytotoxicity against IL-15 overexpressing target cells, resulting in a reduction in IL-15 expressing cells and macrophages in blood and spleen and a decreased CD4/CD8 ratio.
Conclusion: Hypercholesterolemia leads to upregulation of IL-15 within spleen and blood. DNA vaccination against IL-15 does markedly reduces atherosclerotic lesion size, but does not promote lesion stability.

1. Introduction

Atherosclerosis is characterized as a dyslipidemic induced chronic inflammatory disease of the arterial wall [1]. During the various stages of lesion development, monocytes and T cells are recruited to the arterial wall [2], already in the early stages of atherogenesis, macrophages and T cells are present in the intima of the atherosclerotic plaque [3]. Interleukin 15 (IL-15) is a pro-inflammatory cytokine which is expressed by different immune cells such as monocytes and macrophages and promotes T cell proliferation independently of antigen-specific T cell receptor activation [4]. IL-15 is also expressed in a biologically active form on the surface of monocytes and activated macrophages. This surface expressed IL-15 is approximately 5 times more effective than soluble IL-15 in the induction of T cell proliferation [5]. IL-15 expression is associated with chronic inflammatory diseases such as rheumatoid arthritis [6]. In addition, IL-15 is found to be expressed in human and murine atherosclerotic lesions [7,8] and may therefore affect T cells within the plaque.

The IL-15 receptor shares two subunits, the β and γ_c subunit, with the IL-2 receptor, while the third subunit is formed by a unique α-chain, IL-15Rα [9]. Because the IL-15 and IL-2 receptor share two subunits, IL-15 shares biological activities with IL-2, such as the induction of proliferation of T cell subsets. There are however opposing effects of IL-2 and IL-15. IL-2 is primarily involved in the maintenance of regulatory T cells and IL-15 plays mainly a role in the survival of T cells and thus in memory cell formation [10–12].

IL-15 not only activates T cells, it is also a strong chemoattractant for T cells and Natural killer (NK) cells [13,14] and enhances CD44 mediated T cell adhesion to endothelial cells [15]. IL-15 is also involved in expansion and survival of Natural killer T (NKT) cells, which form an important link between the innate and adaptive immune response and enhance atherosclerosis [16]. IL-15 finally exerts an autocrine regulation of the production of pro-inflammatory cytokines by macrophages, such as TNF-α, IL-6 and IL-1β [17].

We studied the role of IL-15 in atherosclerotic lesion formation by applying an *in vivo* blockade of IL-15 using oral vaccination,

* Corresponding author at: Therapeutic Immunomodulation, Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug research, Einsteinweg 55, PO Box 9502, 2300 RA Leiden, The Netherlands. Tel.: +31 715274378; fax: +31 715276032.
E-mail address: ikbenthomasvanes@hotmail.com (J. Kuiper).

0264-410X
doi:10.1016/j.vaccine.2010.11.037

which resulted in a 75% reduction in lesion size with a concomitant increase in macrophage content of the plaque, thereby establishing an important role for IL-15 in atherogenesis.

2. Methods

2.1. Animals, materials, bacterial strains and cell lines

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. The mice were kept under standard laboratory conditions and food and water were provided *ad libitum*. Recombinant murine IL-15 was purchased from PeproTech, biotinylated polyclonal mouse anti-IL-15 was obtained from R&D systems. The attenuated *Salmonella typhimurium* (Dam-;AroA-,strain:SL7207) was provided by Dr. Kriszitana M. Zsebo (Remedyne Corporation, Santa-Barbara, CA). The macrophage cell line(RAW246.7), the endothelial cell line(H5V) and mouse fibroblasts were cultured in DMEM with 10% FCS, 2 mmol/L glutamin, 0.1 U/L penicillin, and 100 mg/L streptomycin. Vascular smooth muscle cells were isolated from a murine aorta and cultured as described previously [18].

2.2. In vitro assessment of IL-15

Cells were added to a 24-well plate (2.5×10^5 RAW cells/mL, 1.0×10^5 cells for H5V and vSMC). Where stated, 100 ng/ml recombinant IL-15 was added to the culturing medium and culturing medium alone served as a control. Cells were incubated for 24 h, and thereafter the cells were used for qPCR and the supernatant was used for ELISA. All experiments were performed in triplicate.

2.3. RNA isolation and qPCR

Total RNA was isolated using Trizol (Boehringer Mannheim) and reverse transcribed (RevertAidP^{TMP} M-MuLV reverse transcriptase, Fermentas). qPCR was analyzed with SYBRgreen mastermix (PerkinElmer) and a final concentration of 300 nM primers (Table 1), using acidic ribosomal phosphoproteinP0(36B4) as an internal standard.

2.4. ELISA

A mouse TNF-α set (PharMingen) was used to detect TNF-α in culture supernatant according to manufacturers' protocol.

2.5. Construction of the DNA vaccine

Murine IL-15 (AI503618) was cloned into the eukaryotic expression plasmid pcDNA3.1 (Invitrogen). The 605 bp. fragment encoding the entire IL-15 gene was amplified using PCR primers: 5′-GAAGCCCATCGCCATAGC-3′ and 5′-GAGCAGCAGGTGGAGGTA-3′ and subsequent cloned into pcDNA3.1 with *Eco*RV, generating pcDNA3.1-IL-15. Subsequently, *S. typhimurium* was electroporated with pcDNA3.1-IL-15 or an empty pcDNA3.1 plasmid [19].

2.6. Vaccination and the induction of atherosclerosis

Mice were vaccinated prior to the induction of atherosclerosis with 10^8 cfu *S. typhimurium* transformed with empty pcDNA3.1 (control) or pcDNA3.1-IL-15 as previously described [19]. Male LDLr$^{-/-}$ mice 10–12 weeks of age were fed a Western-type diet containing 15% cocoa butter and 0.25% cholesterol 2 weeks prior to collar placement. Atherosclerosis was induced by placement of collars (0.3 mm, Dow Corning, Midland, Michigan) around the carotid arteries as previously described [20]. Hereafter, the mice were fed a Western-type diet for 8 more weeks. Total cholesterol levels during the experiment were quantified spectrophotometrically using an enzymatic procedure (Roche Diagnostics, Germany). Precipath standardized serum (Boehringer, Germany) was used as an internal standard.

2.7. Cytotoxicity assay

The murine fibroblast cells were used as target cells and were co-transfected with pcDNA3.1-IL-15 and pcDNA3.1-eGFP using ExGen500 (Fermentas, Germany) according to the manufacturer's protocol. 24 h after transfection, 10^6 spleen cells isolated from IL-15 vaccinated or control vaccinated mice were added to the target cells. 24 h later, cells were fixed using FormalFixx (3.7%, Thermo Shandon, Pittsburgh, PA), and the number of GFP-fluorescent cells per well was determined.

2.8. Histology and immunohistochemistry

Carotid arteries were removed for analysis as described by Von der Thüsen et al. [20]. The arteries were embedded in OCT compound (TissueTek; Sakura Finetek, The Netherlands). Cryosections of 5 μm were made proximally of the collar occlusion and stained with hematoxylin (Sigma Diagnostics, MO) and eosin (Merck Diagnostica, Germany). Corresponding sections on separate slides were stained immunohistochemically for macrophages using an antibody against a macrophage-specific antigen (MoMa-2, Research Diagnostics Inc.). Quantification of the staining was performed by using a Leica DM-RE microscope and Leica Qwin Imaging software (Leica Ltd., Germany).

2.9. FACS analysis of leukocytes

Peripheral Blood Mononuclear Cells (PBMC) were isolated after orbital bleeding using Lympholyte (Cedarlane, Canada) as described in the manufacture's protocol. Spleens were dissected and single cell suspension was obtained by passing the spleen through a 70 μm cell strainer (Falcon, The Netherlands). Leukocytes were purified using Lympholyte. Cells were stained with FITC-conjugated anti-mouse CD8 (0.125 μg/sample, Pharmingen) and PE-conjugated anti-mouse CD69 (0.125 μg/sample, eBioscience). For the staining of surface bound IL-15, the leukocytes were stained with biotinylated anti-mouse IL-15 (R&D systems) and PE-conjugated streptavidin (BD Pharmingen) and analyzed by flow-cytometry on a FACSCalibur. All data was analyzed with CELLQuest software (BD Bioscience, The Netherlands).

2.10. Statistical analysis

All data are expressed as means ± 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.

3. Results

3.1. IL-15 is upregulated in hypercholesterolemic mice

The spleens of LDLr$^{-/-}$ mice were collected at different time points after the start of the Western-type diet feeding and mRNA expression of IL-15 was quantified. The expression of IL-15 mRNA was significantly elevated in the spleen at 6 weeks after the start of the diet (Fig. 1A). Since IL-15 expression is also regulated at a post-translational level and is mainly membrane bound [5], we also determined the cell surface expression of IL-15. Spleen cells and PBMCs were isolated from LDLr$^{-/-}$ mice which were fed a

Table 1
Primer sequences for quantitative PCR.

Gene	GenBank acession #	Forward primer 5′ → 3′	Reverse primer 5′ → 3′	Amplicon size	bp
IL-15	NM_008357	TGAGGCTGGCATTCATGTCTT	ATCTATCCAGTTGGCCTCTGTTTT	75	546–621
IL-1ß	NM_008361	TGGTGTGTGACGTTCCCATTA	AGGTGGAGAGCTTTCAGCTCATAT	102	341–443
IL-10	M37897	TCTTACTGACTGGCATGAGGATCA	GTCCGCAGCTCTAGGAGCAT	105	107–212
CXCL1	NM_008176	GGCGCCTATCGCCAATG	CCTGAGGGCAACACCTTCAA	95	73–168
CCL2	M19681	GCATCTGCCCTAAGGTCTTCA	TTCACTGTCACACTGGTCACTCCTA	127	398–525
CCR2	MMU51717	CCTTGGGAATGAGTAACTGTGTGA	TGGAGAGATACCTTCGGAACTTCT	140	942–1082
36B4	NM_007475	GGACCCGAGAAGACCTCCTT	GCACATCACTCAGAATTTCAATGG	85	464–548

Western diet or a normal Chow diet for 10 weeks. FACS analysis showed that the percentage of IL-15 expressing cells within the spleen and PBMCs was highly elevated after 10 weeks of Western type diet (Fig. 1B; $12.59 \pm 0.65\%$ versus $26.07 \pm 3.44\%$, $P < 0.05$ and $0.28 \pm 0.06\%$ versus $4.95 \pm 0.98\%$, $P < 0.05$, respectively).

3.2. IL-15 predominately affects macrophages in vitro

We determined the effect of IL-15 on cell lines that represent the main cell types in the atherosclerotic lesion; macrophages (RAW cells), vascular smooth muscle cells (vSMCs) and endothelial cells (H5V cells). The relative expression is highest for macrophages (Fig. 2A), while also for vSMCs and endothelial cells a distinct expression is found. Addition of recombinant IL-15 to the various cell types induced only in macrophages an increased expression of tumor necrosis factor (TNF)-α on protein level (Fig. 2B). In line with the increase in TNF-alpha, we observed in macrophages a distinct increase in the pro-inflammatory cytokine IL-1β, whereas there was no significant effect seen on mRNA encoding IL-10 (Fig. 2C), IFN-γ or IL-12 (p40) (data not shown). In addition, IL-15 significantly induced the expression of CXCL1, CCL2 and CCR2 in macrophages (Fig. 2D). These results indicate that IL-15 may affect the chemokines induced migration of macrophages [21]. Endothelial cells did not respond to IL-15 by upregulation of CXCL1, CCL2 or CCR2 on mRNA levels. In addition, IL-15 did not affect the expression of adhesion molecules such as VCAM-1, ICAM-1, PECAM and P-selectin in endothelial cells (data not shown).

3.3. Vaccination against IL-15 induces an IL-15 specific cytotoxic T cell response

The Western-diet induced IL-15 expression on spleen cells and PBMCs and the IL-15 mediated activation of macrophage stimulated us to analyze the effect of IL-15 blockade via vaccination. To this end, LDLr$^{-/-}$ mice were vaccinated against IL-15 by oral delivery using an attenuated strain of *S. typhimurium* transformed with an IL-15 expression vector (pcDNA3.1-IL-15) or with *S. typhimurium* transformed with an empty vector (pcDNA3.1) as a control. This vaccination strategy leads to the induction of CD8$^+$ cytotoxic T cells that specifically lyse those cells that overexpress IL-15 and present IL-15 peptides via MHC-I [19]. This protocol was used to study the role of VEGFR2 and CD99 in atherosclerosis [22,23]. Following vaccination, mice were fed a Western-type diet for 2 weeks and collars were placed around the carotid arteries which results in flow-induced atherosclerotic lesion formation [20]. A Subsequent to vaccination, we established the activation state of the CD8$^+$ T cell population. Spleen cells were isolated and stained for CD8 and CD69, an early T cell activation marker, and the percentage of CD8$^+$CD69$^+$ double positive cells was significantly increased upon vaccination against IL-15 compared to the control vaccination (Fig. 3A; $16.0 \pm 2.1\%$ versus $10.4 \pm 0.1\%$, $P < 0.05$). In order to study the specificity of CD8$^+$ cytotoxic T cells, spleen cells from vaccinated and control mice were co-cultured with murine fibroblasts that were co-transfected with pcDNA3.1-IL-15 and pcDNA3.1-GFP. The number of surviving IL-15 expressing target cells was determined by counting GFP positive cells. The number of IL-15 expressing target cells was reduced by 50% after incubation with spleen cells from IL-15 vaccinated mice, whereas spleen cells from control vaccinated mice, did not significantly lyse IL-15 expressing cells (Fig. 3B; $49 \pm 1\%$ in vaccinated group versus $81 \pm 4\%$ in control group, $P < 0.01$).

3.4. Vaccination against IL-15 reduces atherosclerotic lesion size in hypercholesterolemic LDLr$^{-/-}$ mice

Atherosclerosis was determined in control and IL-15 vaccinated mice 6 weeks after collar placement. IL-15 vaccination did not affect plasma cholesterol levels during the experiment (Fig. 3C).

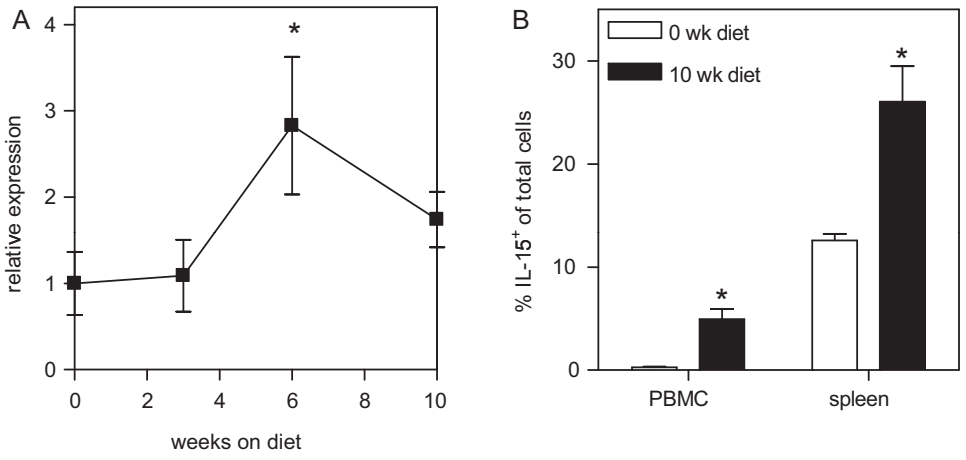

Fig. 1. Expression of IL-15 in hypercholesterolemic mice. IL-15 mRNA expression level was determined in spleen cells of LDLr$^{-/-}$ mice at different time points on Western type diet (A). PBMCs and spleen cells were isolated and stained for surface bound IL-15(B). The percentages of IL-15 positive cells are determined by FACS analysis after 0 weeks of Western type diet (white bars, $N = 5$) and after 10 weeks of Western type diet (black bars, $N = 5$). *$P < 0.05$.

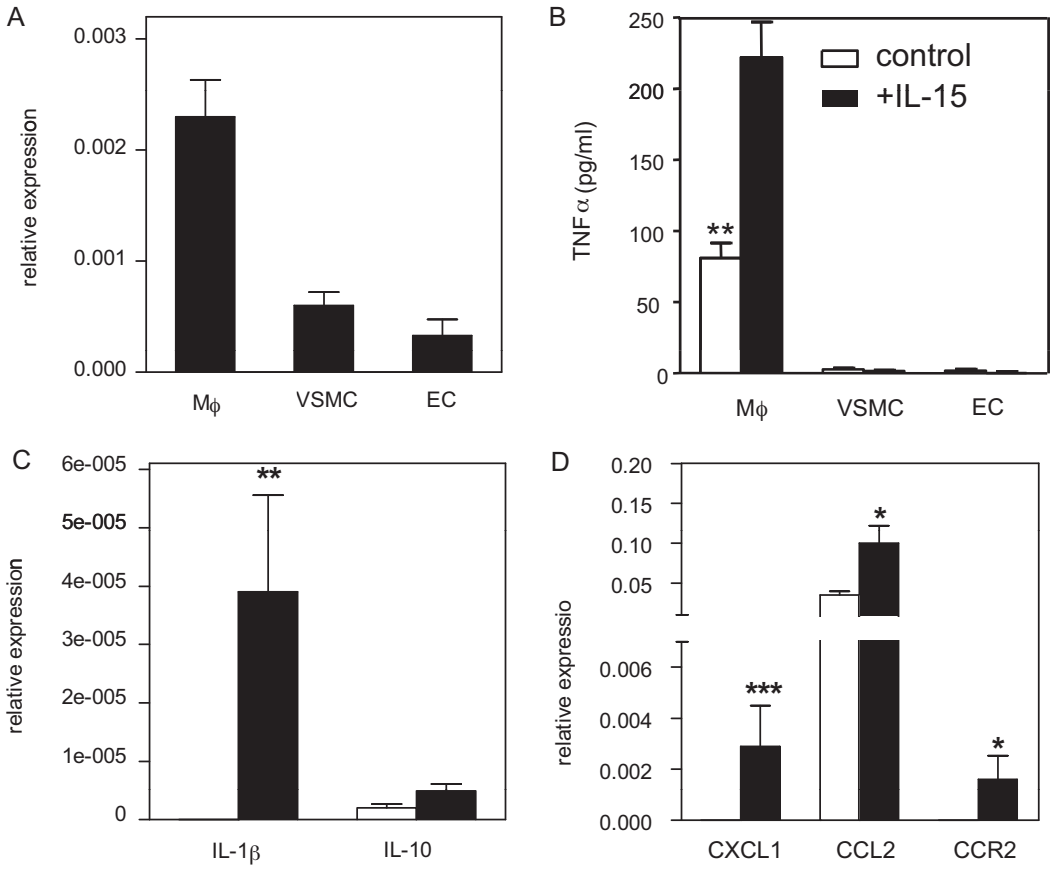

Fig. 2. Effect of IL-15 on gene expression in plaque related cell types. IL-15 expression was determined in RAW cells, H5V cells and vSMC (A). IL-15 (black) or no IL-15 (control; white) was added to different cell types. Cellular activation was measured by TNF-α release (B). The expression of several genes was measured in RAW cells after addition of 100 ng/mL IL-15 (black), or without IL-15 (white) for inflammatory genes (C) and chemotaxic associated genes (D). $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$

Quantification of Hematoxylin–Eosin (HE) stained atherosclerotic plaques showed that vaccination against IL-15 resulted in a 75% decrease in lesion size as compared to the control group (Fig. 4A–C; $13722 \pm 3116\ \mu m^2$ versus $53977 \pm 15332\ \mu m^2$, $P<0.05$). Immunohistochemical staining for macrophages showed a significant change in plaque composition (Fig. 4F). The relative number of macrophages per plaque area was 2-fold higher in IL-15 vaccinated mice (Fig. 4E) than that in control vaccinated mice (Fig. 4D), indicative for a less advanced state of the lesions in the vaccinated mice.

3.5. Surface expression of IL-15 and CD4/CD8 ratio is reduced after vaccination

As hypercholesterolemia induced surface expression of IL-15 on PBMCs and spleen cells (Fig. 1B) we evaluated the effect of IL-15 vaccination on the percentage of IL-15 positive cells within the spleen and PBMCs. Spleen cells and PBMCs were stained for IL-15 and for the macrophage marker F4/80 and analyzed by FACS. Upon IL-15 vaccination, the surface expression of IL-15 on spleen cells was almost completely reduced to a level comparable to that determined in mice before the start of the Western-type diet (Fig. 5A, $P<0.05$). Within the PBMC population IL-15 surface expression was also decreased (Fig. 5A, $P<0.05$). Within the macrophage population we observed an almost 70% reduction in the percentage of IL-15 positive macrophages (Fig. 5B, $P<0.01$), while the CD4/CD8 ratio in blood, indicative of the inflammatoruy status of the mice, was 3-fold lower in the IL-15 vaccinated mice (Fig. 5, $P<0.01$).

4. Discussion

Atherosclerosis is considered a dyslipidemia-induced chronic inflammatory disease of the arterial wall. During atherosclerotic lesion formation, monocytes and subsequently T cells infiltrate the arterial wall [1]. DNA vaccination against IL-15 leads in $LDLr^{-/-}$ mice to a blocked atherosclerotic lesion development, indicating that IL-15 accelerates lesion formation.

Upon the start of a hypercholesterolemic diet in $LDLr^{-/-}$ mice the mRNA expression of IL-15 is increased within the spleen. Furthermore, hypercholesterolemia does increase the percentage of IL-15 expressing cells in both blood and in spleen. These findings point to a possible relation between IL-15 expression and the induction of atherosclerosis. IL-15 appears to be highly expressed by macrophages and to a lesser extend by endothelial cells and vSMCs. After stimulation of macrophages with IL-15, the mRNA level of several pro-inflammatory cytokines, such as TNF-α and IL-1β are upregulated, while the secretion of TNF-α is increased by IL-15. Important proteins in the chemoattraction of macrophages, CXCL1, CCL2 and CCR2, are also upregulated after incubation with IL-15. These latter effects are also seen on human monocytes when stimulated with IL-15 [24].

Vaccination against IL-15 was accomplished by oral administration of a live attenuated *S. typhimurium* bacteria, transformed with an eukaryotic expression vector encoding IL-15. This vaccination method induces a strong, IL-15 specific, cytotoxic immune response, resulting in the killing of cells overexpressing IL-15. This is a similar mechanism as achieved by the oral vaccination against FLK-1 as described by Niethammer et al. [19] and by Hauer et al. [22] and vaccination against CD99 described by

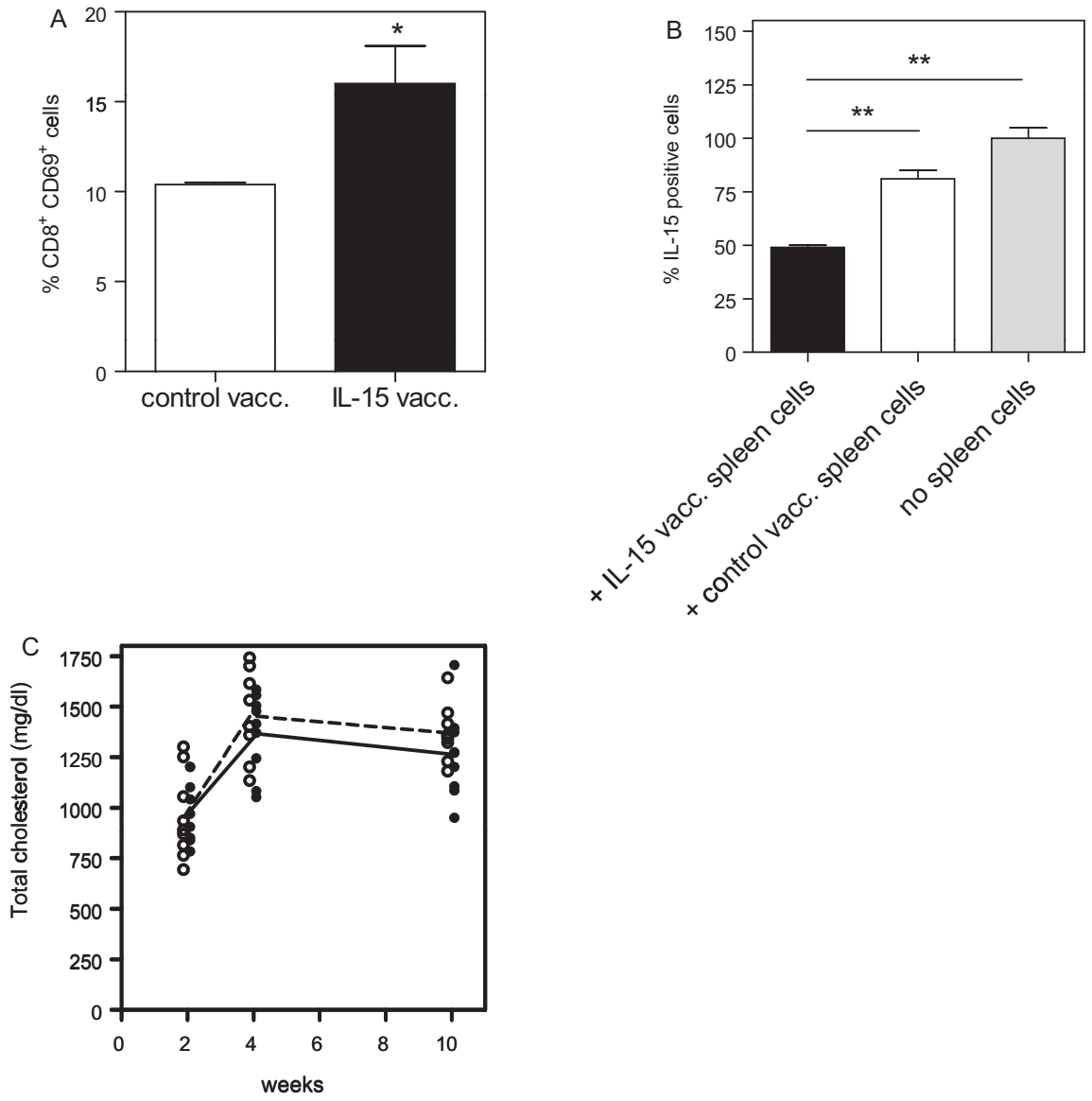

Fig. 3. Effect of the vaccination against IL-15 *in vivo*. The percentage of double positive cells ($CD8^+CD69^+$ cells) was determined in a single cell suspension of the spleen cells in vaccinated and control mice (A, $N = 5$). The induction of specific cytotoxic $CD8^+$ T cells against IL-15 expressing cells was determined by incubating spleen cells from vaccinated mice (black), control mice (white) or without spleen cells (grey) with fibroblast transfected with an IL-15 and GFP expression plasmid (B, $N = 5$). Blood was taken from vaccinated (closed symbols, solid line) and control mice (open symbols, dashed line) at the indicated times and total cholesterol level was determined in serum (C, $N = 9$). $^*P < 0.05$ and $^{**}P < 0.01$

van Wanrooij et al. [23]. These vaccination procedures resulted in a cytotoxic T cell-mediated killing of cells expressing FLK-1 and CD99, respectively. The reduction in IL-15 expressing cells within the spleen and blood upon vaccination was accompanied by a 75% reduction in atherosclerotic lesion size. During the experiment no difference was detected in total serum cholesterol levels between the groups, indicating that IL-15 does not affect lipid-metabolism and the reduction in plaque is more likely due to changes in the inflammatory status of the mice, similar to previous studies in which lowering the inflammatory status reduced atherosclerosis without affecting cholesterol levels [29]. The reduced plaque size was accompanied by a two-fold increase in the relative amount of macrophages. As macrophage infiltration is a feature of early vascular lesion formation [25], it may be speculated that plaque formation and progression is strongly retarded but not prevented due to the blocking of IL-15. In addition, it is clear that the smaller lesion tat develops upon IL-15 vaccination is more vulnerable since the macrophage content is higher and the increased plaque instability after IL-15 vaccination is in contrast to previous experiments of our group which in IL-12 vaccination both reduced the plaque size and improved the stability of the plaque [29].

Although, IL-15 is involved in the expression of important chemoattractants for macrophages it is likely that there are additional sources for these chemokines within the plaque, for example endothelial cells or vSMCs. We can also speculate that the recruited macrophages within the plaque do not, or to a lesser extent, express IL-15/IL15Rα as is demonstrated by the reduction of the surface expression of IL-15 on cells within spleen and PBMCs. Macrophages express IL-15/IL15Rα complexes on their surface upon activation and are able to activate T cells in an antigen-independent way. Membrane bound IL-15 is not only 5-times more effective in inducing T cell proliferation than soluble

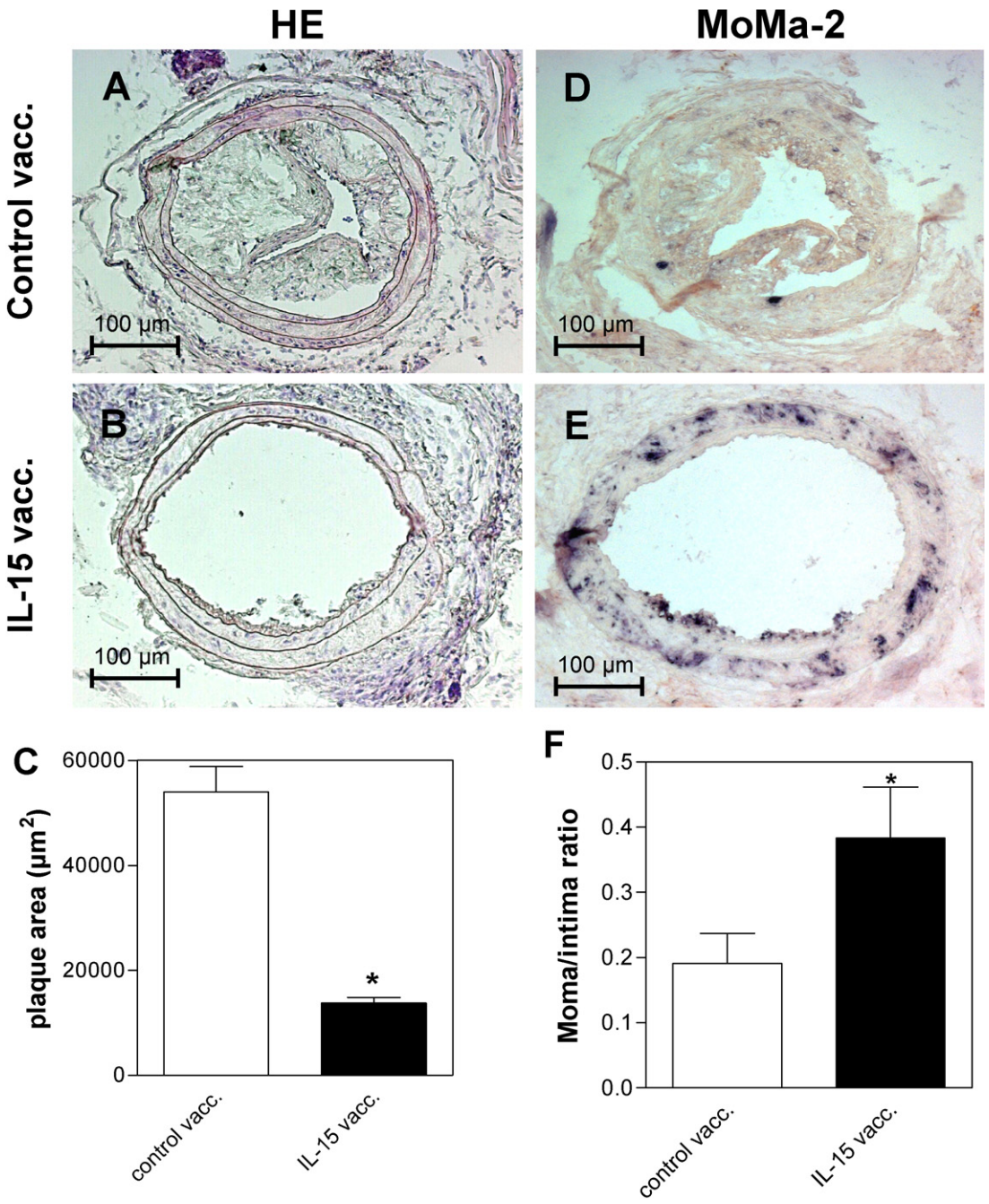

Fig. 4. The effects of vaccination on atherosclerotic lesion development in LDLr$^{-/-}$ mice. 5 μm cross sections of the carotid artery of the control group (A) and the vaccinated group (B) were made and subsequently stained for HE and quantified (C, $N = 8$). To determine macrophage content, cross sections were made and stained with MoMa-2 (D and E) and the ratio of macrophage count and plaque area was determined (F, $N = 8$). *$P < 0.05$

IL-15, it also signals through different effectors and can therefore exert distinct biological responses. Membrane bound IL-15 expressed on macrophages can participate in reverse signaling between the IL-15Rα on T cells, whereas soluble IL-15 modulates cellular function in both a paracrine and autocrine fashion [17,26]. Macrophages which lack IL-15/IL15Rα complex on the surface are not able to sustain a full immune response within the plaque and thereby are less capable to recruit inflammatory cells into the plaque, which is reflected in the reduced CD/CD8 ratio, indicative of a lower inflammatory status, after IL-15 vaccination.

We suggest that the development of the lesion is arrested in the fatty streak stadium. This may provide an explanation for the increased number of macrophages in the vessel wall and the smaller lesion size, since mainly the innate immune response is activated and adaptive immune response is likely impaired. However, IL-15 expressing cells are activated inflammatory cells, which are also able to express other inflammatory mediators. Therefore it should be taken into account that the effect we observe may also be due to the absence of other mediators. The vaccination method used in this study may lead to the initiation of new therapies, which block the action of IL-15. There are some promising results with phase I/II clinical trails with an anti-IL-15 antibody treatment in patients with rheumatoid arthritis [27], which might be extended to cardiovascular patients. Furthermore Gokkusu et al. [28], recently demonstrated that genetic variation in IL-15 gene and

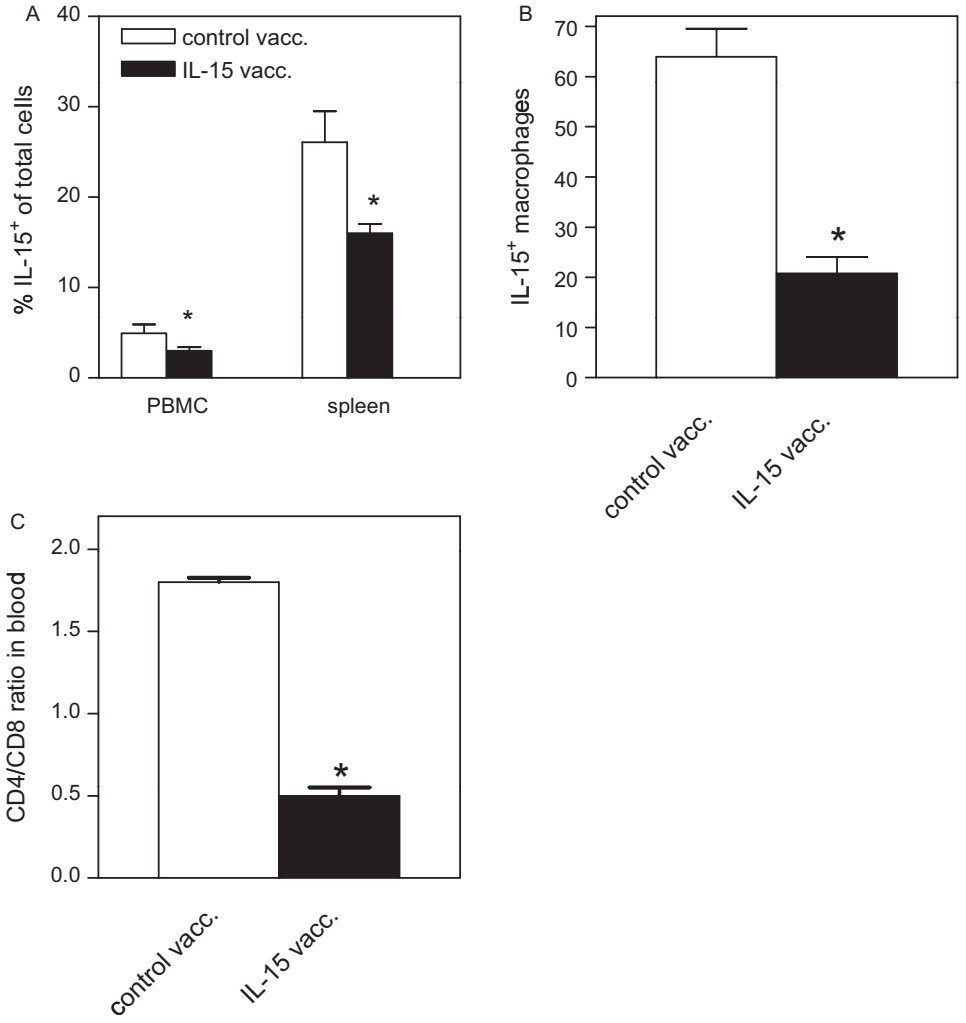

Fig. 5. Leukocyte IL-15 expression in vaccinated and control LDLr$^{-/-}$ mice. After vaccination and collar placement, PBMCs and spleen cells were isolated and stained for IL-15, F4/80 and CD4/CD8. The percentage of IL-15 positive cells in PBMC and spleen cells is determined by FACS analysis, after control vaccination and 10 weeks of diet (white, $N = 6$), and after IL-15 vaccination and 10 weeks of diet (black, $N = 6$) $^{*}P < 0.05$ and $^{**}P < 0.01$. (A) Percentage of IL-15 positive cells in spleen and PBMC. (B) Percentage of IL-15 positive cells within the macrophage population. (C) Ratio between CD4 and CD8 positive T cells within the blood.

IL-15 levels influence the risk of coronary heart disease, indicating the importance of IL-15 signaling in atherosclerosis.

The vaccination strategy used in this study successfully evoked a chemotoxic response targeting IL-15 expressing cells. This resulted in a vast reduction in atherosclerosis, thereby providing new insights in the process of atherosclerosis and the contribution of IL-15 in this process. These new insights may contribute to the future immunomodulating treatment of patients with cardiovascular diseases.

Acknowledgement

Johan Kuiper is an established investigator from the Netherlands Heart Foundation (grant 2000T040) and Gijs H.M. van Puijvelde is a postdoctoral fellow of the Netherlands Heart Foundation (2007T039).

References

[1] Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340(2):115–26.
[2] Hansson GK, Robertson AK, Soderberg-Naucler C. Inflammation and atherosclerosis. Annu Rev Pathol 2006;1:297–329.
[3] Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci USA 1995;92(9):3893–7.
[4] Liu K, Catalfamo M, Li Y, Henkart PA, Weng NP. IL-15 mimics T cell receptor crosslinking in the induction of cellular proliferation, gene expression, and cytotoxicity in CD8+ memory T cells. Proc Natl Acad Sci USA 2002;99(9):6192–7.
[5] Dubois S, Mariner J, Waldmann TA, Tagaya Y. IL-15Ralpha recycles and presents IL-15 In trans to neighboring cells. Immunity 2002;17(5):537–47.
[6] Yoshihara K, Yamada H, Hori A, Yajima T, Kubo C, Yoshikai Y. IL-15 exacerbates collagen-induced arthritis with an enhanced CD4+ T cell response to produce IL-17. Eur J Immunol 2007;37(10):2744–52.
[7] Houtkamp MA, van Der Wal AC, de Boer OJ, van Der Loos CM, de Boer PA, Moorman AF, et al. Interleukin-15 expression in atherosclerotic plaques: an alternative pathway for T-cell activation in atherosclerosis? Arterioscler Thromb Vasc Biol 2001;21(7):1208–13.
[8] Wuttge DM, Eriksson P, Sirsjo A, Hansson GK, Stemme S. Expression of interleukin-15 in mouse and human atherosclerotic lesions. Am J Pathol 2001;159(2):417–23.
[9] Giri JG, Anderson DM, Kumaki S, Park LS, Grabstein KH, Cosman D. IL-15, a novel T cell growth factor that shares activities and receptor components with IL-2. J Leukoc Biol 1995;57(5):763–6.
[10] Waldmann TA, Dubois S, Tagaya Y. Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy. Immunity 2001;14(2):105–10.

[11] Ku CC, Murakami M, Sakamoto A, Kappler J, Marrack P. Control of homeostasis of CD8+ memory T cells by opposing cytokines. Science 2000;288(5466):675–8.
[12] Zhang X, Sun S, Hwang I, Tough DF, Sprent J. Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. Immunity 1998;8(5):591–9.
[13] Allavena P, Giardina G, Bianchi G, Mantovani A. IL-15 is chemotactic for natural killer cells and stimulates their adhesion to vascular endothelium. J Leukoc Biol 1997;61(6):729–35.
[14] Wilkinson PC, Komai-Koma M, Newman I. Locomotion and chemotaxis of lymphocytes. Autoimmunity 1997;26(1):55–72.
[15] Estess P, Nandi A, Mohamadzadeh M, Siegelman MH. Interleukin 15 induces endothelial hyaluronan expression in vitro and promotes activated T cell extravasation through a CD44-dependent pathway in vivo. J Exp Med 1999;190(1):9–19.
[16] Matsuda JL, Gapin L, Sidobre S, Kieper WC, Tan JT, Ceredig R, et al. Homeostasis of V alpha 14i NKT cells. Nat Immunol 2002;3(10):966–74.
[17] Alleva DG, Kaser SB, Monroy MA, Fenton MJ, Beller DI. IL-15 functions as a potent autocrine regulator of macrophage proinflammatory cytokine production: evidence for differential receptor subunit utilization associated with stimulation or inhibition. J Immunol 1997;159(6):2941–51.
[18] Michon IN, Hauer AD, von der Thusen JH, Molenaar TJ, van Berkel TJ, Biessen EA, et al. Targeting of peptides to restenotic vascular smooth muscle cells using phage display in vitro and in vivo. Biochim Biophys Acta 2002;1591(1–3):87–97.
[19] Niethammer AG, Xiang R, Becker JC, Wodrich H, Pertl U, Karsten G, et al. A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. Nat Med 2002;8(12):1369–75.
[20] von der Thusen JH, van Berkel TJ, Biessen EA. Induction of rapid atherogenesis by perivascular carotid collar placement in apolipoprotein E-deficient and low-density lipoprotein receptor-deficient mice. Circulation 2001;103(8):1164–70.
[21] Cybulsky MI, Gimbrone Jr MA. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 1991;251(4995):788–91.
[22] Hauer AD, van Puijvelde GH, Peterse N, de Vos P, van Weel V, van Wanrooij EJ, et al. Vaccination against VEGFR2 attenuates initiation and progression of atherosclerosis. Arterioscler Thromb Vasc Biol 2007;27(9):2050–7.
[23] van Wanrooij EJ, de Vos P, Bixel MG, Vestweber D, van Berkel TJ, Kuiper J. Vaccination against CD99 inhibits atherogenesis in low-density lipoprotein receptor-deficient mice. Cardiovasc Res 2008;78:590–6.
[24] Badolato R, Ponzi AN, Millesimo M, Notarangelo LD, Musso T. Interleukin-15 (IL-15) induces IL-8 and monocyte chemotactic protein 1 production in human monocytes. Blood 1997;90(7):2804–9.
[25] Hansson GK, Jonasson L, Seifert PS, Stemme S. Immune mechanisms in atherosclerosis. Arteriosclerosis 1989;9(5):567–78.
[26] Musso T, Calosso L, Zucca M, Millesimo M, Ravarino D, Giovarelli M, et al. Human monocytes constitutively express membrane-bound, biologically active, and interferon-gamma-upregulated interleukin-15. Blood 1999;93(10):3531–9.
[27] Baslund B, Tvede N, Danneskiold-Samsoe B, Larsson P, Panayi G, Petersen J, et al. Targeting interleukin-15 in patients with rheumatoid arthritis: a proof-of-concept study. Arthritis Rheum 2005;52(9):2686–92.
[28] Gokkusu C, Aydin M, Ozkok E, Tulubas F, Elitok A, Pamukcu B, et al. Influences of genetic variants in interleukin-15 gene and serum interleukin-15 levels on coronary heart disease. Cytokine 2010;49(1):58–63.
[29] Hauer AD, Uyttenhove C, de Vos P, Stroobant V, Renauld JC, van Berkel ThJC, et al. Blockade of interleukin-12 function by protein vaccination attenuates atherosclerosis. Circulation 2005;112:1054–62.