The potential use of dendritic cells in mouse models of atherosclerosis

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Chapter 6

Foam cell formation affects MHC class I processing and presentation *in vitro* while hypercholesteremia induces a DC-like phenotype in macrophages *in vivo*

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Abstract

Macrophage foam cell formation can induce a differentiation of macrophages into a dendritic cell-like phenotype. Because dendritic cells are the most potent antigen presenting cells, these changes could have implications in antigen presentation and subsequent T cell activation. To evaluate the effects of foam cell formation we have cultured bone-marrow derived macrophages in the presence of oxLDL (10 μg/ml) or acLDL (20 μg/ml). We observed a dendritic cell like phenotype with increased CD11c, MHC-I, MHC-II and costimulatory molecules suggesting an increased capacity to induce a specific T cell response. Consequently, we showed that MHC-I antigen presentation and processing of ovalbumin was increased while MHC-II induced T cell activation was not altered. Next we evaluated the effects of hypercholesteremia on peripheral macrophages *in vivo* in $LDLr^{-/-}$ mice. Again we observed an increase of $CD11c^{high}$ and MHC-I within the macrophage population. Furthermore, the activation status of both dendritic cells as macrophages was increased. In conclusion, both hypercholesteremic conditions as *in vitro* foam cell formation not only induced a dendritic-cell like phenotype in macrophages, but also provide macrophages with dendritic-cell like capacities. These data support the hypothesis that foam cell macrophages could play an important role in inducing an immune response during the early stages of atherosclerotic lesion development.

Introduction

Atherosclerosis is a multifactorial, chronic, auto-immune-like disease initiated by a combination of lipid accumulation and inflammatory processes.[1-3] Key processes in the development of atherosclerosis are the recruitment of inflammatory cells into the arterial wall and subsequent uptake of cholesterol by monocyte-derived macrophages. Cholesterol uptake is tightly regulated through the feedback regulation of the low-density lipoprotein (LDL) receptor. However oxidized LDL (oxLDL) is bound and internalized via a wide arrange of scavenger receptors which leads to the uncontrolled uptake of cholesterol. An ineffective cholesterol output leads to cholesterol accumulation and the formation of lipid-rich foam cells, which are characteristic of an atherosclerotic plaque.[4, 5]

Dendritic cells (DCs) are the most potent antigen presenting cells with the capacity to survey the body for pathogens and to induce and regulate the immune response.[6] As to their role in atherosclerosis, the presence of DCs has been reported in both healthy human and mouse aortic intima and their numbers increase during lesion progression.[7, 8] Evidence is accumulating that foam cell formation induces a dendritic cell-like phenotype in macrophages with enhanced CD11c and MHC-II expression.[9, 10] CD11c is characteristically expressed at high levels by DCs but not by monocytes/macrophages. Hypercholesteremic conditions induce CD11c expression in blood monocytes[11] and the increased CD11c (integrin) expression contributes to the migration of monocytes into the arterial wall of hyperlipidemic mice. In addition oxLDL enhances the adhesion and transmigration of DCs mediated by ICAM-1 and PECAM-1, respectively.[12] Recently, Wu *et al.* reported that under hypercholesteremic conditions, the uptake of modified LDL leads to the formation of $CD11c^+$ foamy macrophages which express high levels of VLA-4.[11] Together these data suggest that under hypercholesteremic conditions, monocytes become more activated, more dendritic cell-like and can migrate more easily into the sub-intimal space; which are all key processes in the progression of atherosclerosis.

However, at present it is unclear whether the induction of a dendritic cell-like phenotype in macrophage-derived foam cells affects their antigen presentation. The aim of this study was therefore to asses the effect of foam-cell formation in bone-marrow derived MΦ and DCs and in macrophages isolated from hypercholesteremic mice. We not only determined the changes in the expression of CD11c and costimulatory molecules upon foam-cell induction but also determined the effect thereof on antigen uptake and presentation and the subsequent capacity to activate T cell subsets.

Materials & methods

Animals

C57BL/6J mice were from Charles River Laboratories. OTII mice were a kind gift from J.M.M. den Haan (Dept. Molecular Cell Biology and Immunology, VUMC, Amsterdam). Mice were kept under standard laboratory conditions and were fed a normal chow diet or a Western-type diet containing 0.25% cholesterol and 15% cocoa butter (Special Diet Services, Witham, Essex, UK). Diet and water were administered *ad libitum*.

Cell lines and cell culture

Cell culture medium was IMDM (PAA, Germany) supplemented with 8 % FCS (PAA, Germany), 100 U/ml streptomycin/penicillin (PAA, Germany), 2 mM glutamax (Invitrogen, The Netherlands) and 20 μM β-mercaptoethanol (cIMDM). The murine T cell hybridoma B3Z is specific for the ovalbumin peptide SIINFEKL in the context of H-2K^{b} and harbors the β-galactosidase gene under the control of the IL2 promoter. B3Z cells were cultured in cIMDM supplemented with hydrochromycin B (500 μg/ml) to select LacZ expressing cells. The Ova-specific, class II-restricted murine T cell hybridoma D011.10 is cultured in cIMDM.

Generation and injection of BM-DC

Bone-marrow derived cells of C57BL/6 mice were harvested and cultured during 10 days in cIMDM in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) to obtain immature/non-foam cell DCs. Purity was assessed by flow cytometry using CD11c as a specific DC marker (CD11c^{high} > 90%).
To obtain macrophages, bone-marrow cells from C57BL/6 mice were resuspended in RPMI supplemented with 20% FCS and 30% of L929-conditioned medium (source for monocyte-colony-stimulating factor, M-CSF). After 7 days of culture, macrophages purity was tested by F4/80 expression (F4/80^{high}>90%).

Isolation of macrophages

Macrophages were isolated from male LDLr$^{-/-}$ mice that have been on a western-type diet for 10 weeks or a normal chow diet. Peritoneal macrophages were isolated by lavage of the peritoneal cavity without prior thioglycolate treatment to avoid activation. CD11b^{+} cells were positively isolated from spleen using magnetically labeled beads following manufacturer's protocol (BD Biosciences, Belgium). Purity of the isolated macrophages was analyzed using flow cytometry (CD11b^{+}>70%).

Antigen-presentation:
MHC class I and class II -restricted proliferation assay

Bone-marrow derived DCs or MΦ were cultured in the presence of medium (control), oxLDL (10 µg/ml) or acLDL (20 µg/ml) during 24 hours to obtain foam cells. APCs were than transferred to 96 well plates (50.000 cells/well) and cultured during 24 hours in the presence of various concentrations of SIINFEKL (0.001; 0.01; 0.1 ng/ml), OVA17 (0.001; 0.01; 0.1 µg/ml) or soluble ovalbumin (Grade V, Sigma-Aldrich, The Netherlands) (0.1; 1; 10 µg/ml) (n=5 per condition). After incubation, 100 µl medium was carefully removed and 100 µl B3Z cells or D011.10 cells were added ($1x10^6$ cells/ml). After 24 hours the medium of the B3Z cells was replaced with 100 µl lysis buffer (phosphate-buffered saline [PBS], 100 µM 2-mercaptoethanol, 9 mM $MgCl_2$, 0.125% NP-40, and 0.15 mM chlorophenol red-ß-D-galactopyranoside) per well. After 3 hours absorbance was read at 590 nm. Values are corrected for the baseline proliferation of T cells in the presence of OVA peptides without APC. The supernatant of the D011.10 cells were collected and IL-2 concentrations were determined using ELISA. Because D011.10 cells are on a BALBc background we added an extra control in which D011.10 cells were co-cultured with non-foam cell APC to rule out the effect of an allogenic reaction. In some experiment proliferation was determined by ^3H thymidine-incorporation. Cultures were pulsed an additional 16 hours with 1 µCi/well of ^3H-thymidine (Amersham Biosciences, The Netherlands) and the amount of ^3H-thymidine incorporation was measured using a liquid scintillation analyzer (Tri-Carb 2900R) as the number of disintegrations per minute (DPM).

CSFE-labeled T cell proliferation

T cells from OTII mice were isolated from spleen and lymph nodes. In short, lymphocytes were selected using a density gradient (Lympholyte, Cederlane). Subsequently, T cells were isolated using the T cell Enrichment Kit (BD Biosciences, Belgium) following manufacturer's instructions. Purity of the obtained T cells was measured with flow-cytometry (CD3>95%). T cells were labeled with CSFE (5 µM) for 15 minutes at 37°C. After washing, 10.000 CSFE-labeled T cells were added to the APCs and after 4 days proliferation was measured using flow-cytometry. The mean fluorescent intensity of CSFE was divided according the number of cell divisions and the percentage of cells within the MFI region for 5 cell cycles was analyzed.

Visualization of ovalbumin uptake by APCs using DQ-ovalbumin

DCs/MΦ were incubated with DQ-ovalbumin-immune complexes with concentrations ranging from 300 µg/ml-0.3 µg/ml (Molecular Probes, The Netherlands) during 1 hour at 37°C. After extensive washing, cells were treated with an acid wash (0.15 M NaCl, 50 mM glycine, 0.1% BSA, pH=2.2) to remove extracellular DQ-ovalbumin. Cytospins of the APCs were obtained by centrifuging cells for 5 min at 500 rpm (Thermo Shandon Cytospin 4). After centrifugation, slides were fixed for 30 minutes using zinc formal Fixx (Shandon, Pittsburgh, USA). Nuclei were counterstained with DAPI (0.2 µg/ml) for 20 minutes. Slides were embedded in Drako Fluorescent Mounting Medium (Dako, VS). Fluorescent microscopy was performed on the Nikon Eclipse E600 microscope. Uptake of the fluorescent DQ-ovalbumin was also checked with flow-cytometry.

Antigen uptake and presentation capacities of lipid-loaden APCs: Ovalbumin-specific proliferation

DCs/MΦ were incubated with various concentrations of ovalbumin ranging from 300 µg/ml-0.3 µg/ml. To further enhance the uptake, ovalbumin-immune complexes (OVA-IC) were generated by incubating soluble ovalbumin with 25 µg/ml polyclonal OVA-specific rabbit IgG (rIgGαOVA; Sigma-Aldrich, The Netherlands) during 30 minutes at 37° C. As a control, soluble ovalbumin was incubated with 25 µg/ml control rabbit IgG. APCs were incubated with OVA or OVA-IC during 24 hours after which class I/II specific proliferation was determined using B3Z/OTII cells or D011.10, respectively.

Statistical analyses

Values are expressed as mean ± SEM. Data were analyzed with either a parametric or non-parametric ANOVA when comparing three groups followed with Turkey post T-testing. When comparing two groups, a two-tailed Student's t-test or Mann-Withey U test was used. Statistical analyses were performed using the Instat3 software. Probability values of $P<0.05$ were considered significant.

Results

Lipid-loading induces a DC-like phenotype in MΦ

After culture, we checked the purity of the obtained bone-marrow derived MΦ and DCs. DCs were more than 90% positive for CD11c and showed an intermediate expression of F4/80. In contrast, MΦ expressed intermediate levels of CD11c but were more than 90% positive for F4/80 (data not shown). Foam cell formation was induced by culture in the presence of oxLDL (10 µg/ml) or acLDL (20 µg/ml). These concentrations induced foam cell formation with low levels of cell death as determined by flow cytometry (Figure 1).

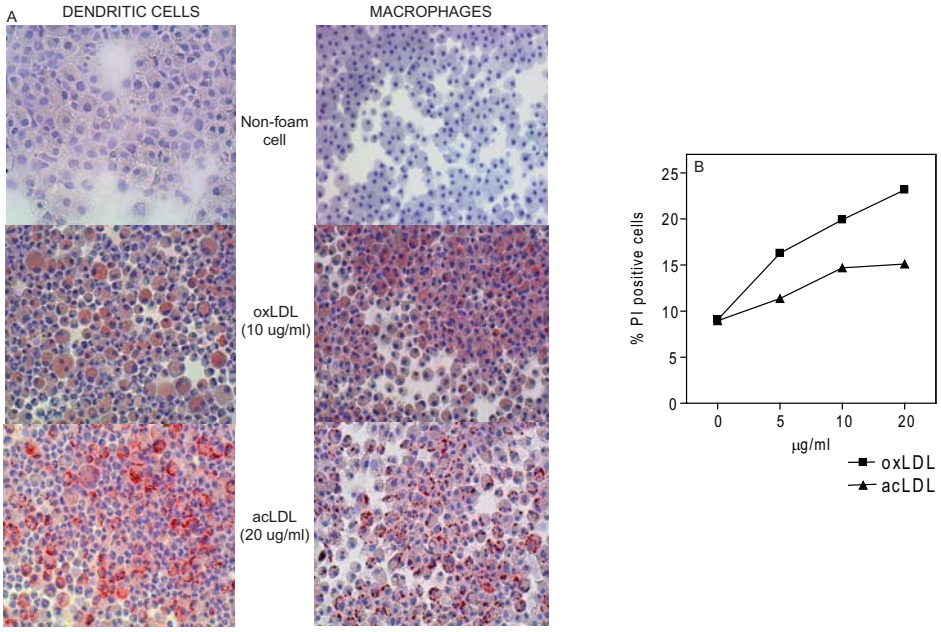

Figure 1: Addition of oxLDL or acLDL to bone-marrow derived DC and MΦ
Bone-marrow derived DCs and MΦ were incubated in the presence of various concentrations oxLDL or acLDL for 24 hours. A. Oil-Red-O staining visualises lipid accumulation. B. Cell death was determined using propidium iodide.

The level of CD11c was increased in MΦ by foam cell formation to reach the CD11c expression level of DCs (Figure 2A) while cholesterol loading in DCs did not affect their level of CD11c expression (Figure 2E). In addition, the level of MHC-I (Figure 2B) and MHC-II (Figure 2C) increased after cholesterol loading in MΦ while only MHC-II expression was higher in DC-derived foam cells (Figure 2F and 2G). Moreover, the expression of the maturation marker CD86 was increased in both MΦ- and DC-derived foam cells (Figure 2D and 2H). Although there was no difference in CD40 and CD80 expression between DCs and MΦ (data not shown), we did observe a higher expression of CD86 and MHC-II in DCs compared to MΦ suggesting that DCs are the better antigen-presenting cells (Figure 2D and 2H).

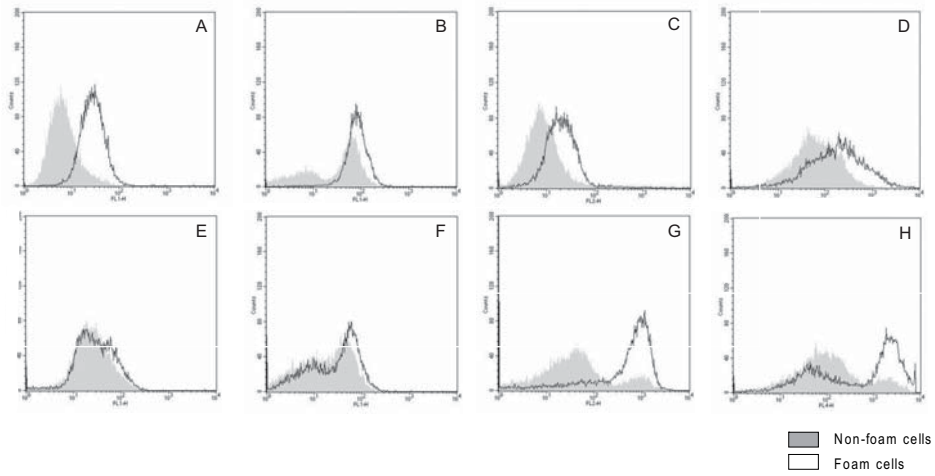

Figure 2: Foam cell formation in MΦ induces a DC-like phenotype
Expression level of several molecules was determined by flow cytometry in macrophages (A-D) and dendritic cells (E-H). CD11c expression (A) and MHC I (B) is increased in foam cell MΦ but is unchanged in foam cell DCs. In addition foam cell formation increased the expression of MHC II (C and G) and CD86 (D and H) in both MΦ and DCs.

Class I antigen presenting capacity of foam cells in vitro

Since lipid loading resulted in an increase in MHC expression, we determined the antigen presenting capacity of lipid loaden macrophages. We incubated MΦ with the MHC class I peptide SIINFEKL after cholesterol loading with either oxLDL or acLDL and determined OVA-specific proliferation. At low concentrations of SIINFEKL, there was no effect of acLDL loading. However, at higher concentrations there was a concentration dependent increase in proliferation of B3Z cells that recognize ovalbumin derived peptides presented by MHC-I (Figure 3A). The same dose response effects were observed after oxLDL loading (Figure 3B), which shows that MΦ-derived foam cells are better APCs than non-foam cell MΦ. The next question was whether lipid-loaded MΦ, which have increased costimulatory molecules, high expression of CD11c, higher expression of MHC-I, are equally potent APC as DCs. To this end, we cultured both DCs and MΦ in the presence of oxLDL (10 µg/ml) or acLDL (20 µg/ml) and determined the MHC-I dependent proliferation of B3Z cells. Again we observed an increased proliferation after lipid-loading of MΦ at the highest concentration of SIINFEKL (Figure 3C). DC-derived foam cells induced proliferation at lower concentrations of SIINFEKL than mΦ-derived foam cells(Figure 3D). Comparing DCs and MΦ we observed that DCs also after lipid loading are in all conditions more competent APCs then MΦ.

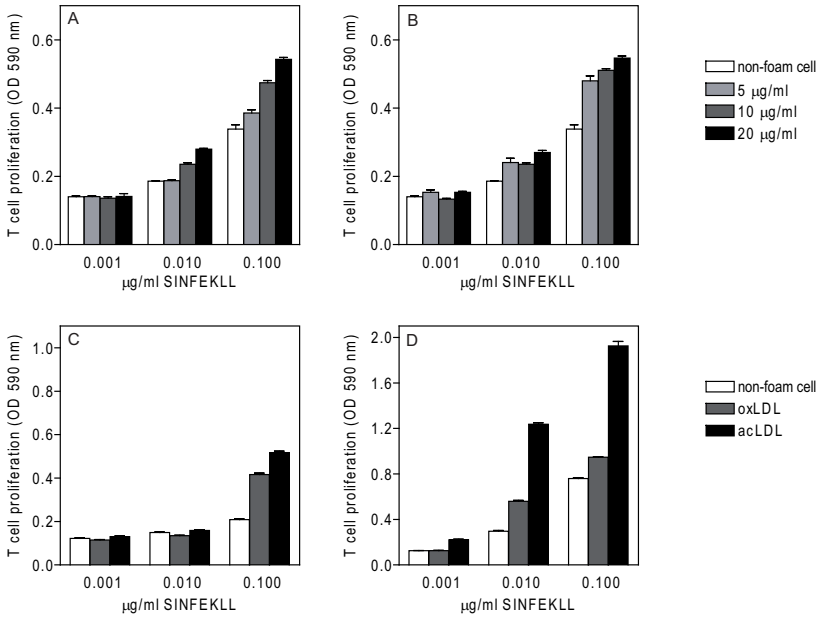

Figure 3: Incubation of APCs with oxLDL or acLDL enhances MHC-I presentation
Bone-marrow derived MΦ were incubated for 24 hours with various concentrations of oxLDL or acLDL. After foam cell formation, various concentrations of SIINFEKL were added and MHC-I specific antigen processing was determined using B3Z cells. We observed a concentration dependent effect of acLDL (A) or oxLDL (B) treatment on T cell proliferation. Next we compared MΦ (C) to DCs (D) after they have been cultured in the presence of oxLDL (10 µg/ml) or acLDL (20 µg/ml). Antigen-specific proliferation was increased after foam-cell formation in both cell types but DCs are better inducers then MΦ.

Antigen processing and MHC-I presentation capacity of foam cells *in vitro*

The previous experiments were performed with ovalbumin derived peptides that can directly bind to MHC-I and do not have to be taken up and processed by the antigen presenting cells. Since foam cell formation may affect antigen uptake and processing, we cultured APCs in different conditions with ovalbumin or with ovalbumin-Ab immune complexes (OVA-IC). Ovalbumin uptake by DCs and MΦ was tested determining the uptake of fluorescently-labeled OVA (DQ-OVA). APCs have taken up the DQ-OVA as shown with fluorescent imaging (Figure 4A) and by flow cytometry (Figure 4B) in a concentration dependent manner. Foam cell formation did not affect the uptake of DQ-OVA in both DCs (Figure 4B and 4C) and MΦ (Figure 4D and 4E).

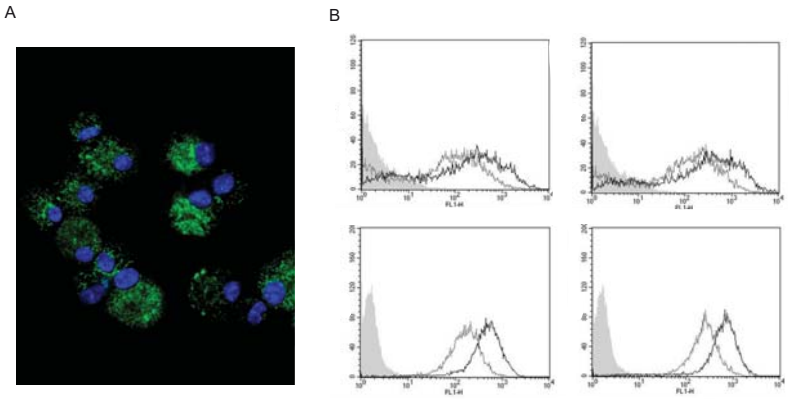

Figure 4: Foam-cell formation does not affect uptake of DQ-ovalbumin
DQ-ovalbumin uptake was visualized by fluorescent microscopy (A). Histograms showing the uptake of increasing concentrations of DQ-ovalbumin by non-foam cell DC (B), DC treated with oxLDL (10 µg/ml) (C); non-foam cell MΦ (D) or MΦ treated with oxLDL (10 µg/ml) (E). Grey curve: 0 ug/ml; grey line: 1 µg/ml; black line; 10 µg/ml DQ ovalbumin.

Next we determined the antigen processing capacities of non-foam cell, LPS-stimulated and MΦ- and DC-derived foam cells. Foam cell formation did not negatively affect ovalbumin antigen presentation in DCs (Figure 5A). In contrast, ovalbumin-specific proliferation was increased in MΦ-derived foam cells (Figure 5B).

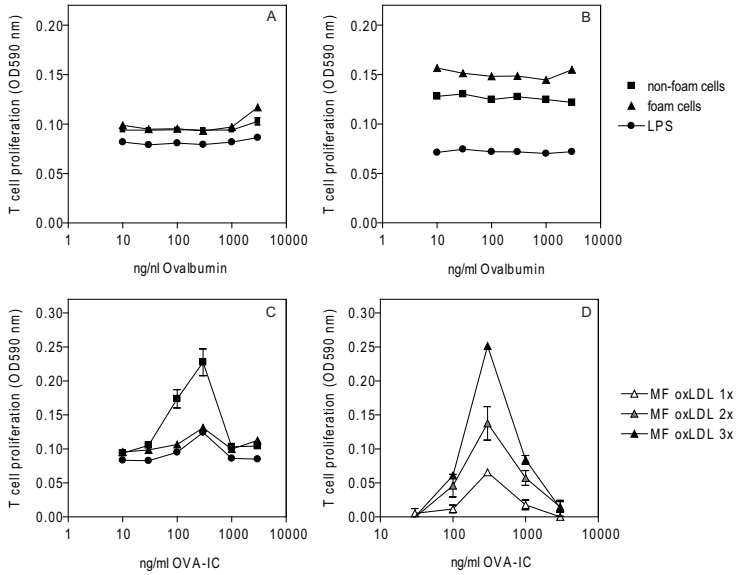

Figure 5: MHC-I specific antigen processing and presentation is enhanced after foam cell formation in macrophages
Bone-marrow derived DCs and MΦ were incubated with ovalbumin (A and B, respecively) or OVA-IC (C and D, respectively) after foam cell formation or LPS stimulation. T cell proliferation was not affected in DCs after foam-cell formation (A) while the activation of specific T cells was decreased after OVA-IC (C). In contrast, proliferation after ovalbumin processing was increased in foam-cell macrophages (B). Increasing amounts of immune-complexes enhanced MHC-I presentation in foam-cell macrophages (D). Values depicted in D are corrected for the amount of proliferation of corresponding non-foam cells.

To further study this observation, we generated ovalbumin-immune complexes (OVA-IC). At the optimal concentration for OVA-IC presentation (300 ng/ml) we analyzed the effect of foam cell formation on antigen processing and presentation. We observed a lowered proliferation of OVA-responsive classI dependent B3Z cells when they are stimulated with DC-derived foam-cells in comaprison to control DCs (Figure 5C). This is in contrast to MΦ-derived foam cells, which induced an increased ovalbumin specific proliferation compared to control MΦ with a optimal response at 300 ng/ml (Figure 6). Next we added larger amounts of immune complexes to further enhance this effect. We observed an increased proliferation of B3Z cells in mΦ-derived foam cells which was correlated to the amount of immune complexes added (Figure 5D). Values were corrected for the amount of proliferation of non-foam cells incubated with increasing amounts of immune-complexes.

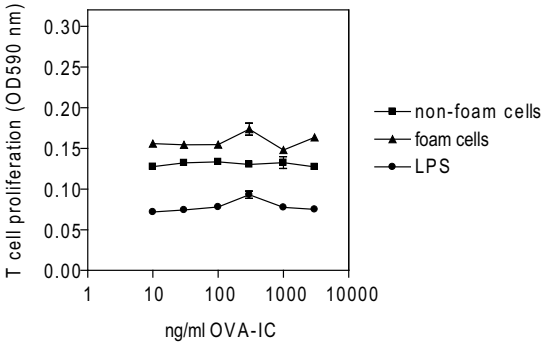

Figure 6: MHC-I specific proliferation is enhanced in macrophage-derived foam cells
Bone-marrow derived MΦ were incubated with OVA-IC after foam cell formation or LPS stimulation and proliferation of B3Z cells was assessed.

Class II antigen presenting and processing capacity of foam cells *in vitro*

To evaluate whether lipid-loading affected MHC-II presentation, we incubated MΦ and DCs with the class II peptide OVA17 and determined OVA-specific proliferation using ova-responsive D011.10 cells. In contrast to the MHC-I peptide SIINFEKL, OVA-17 induced proliferation was not altered after loading of MΦ or DCs with increasing concentrations of acLDL or oxLDL (Figure 7A and 7B). Again, DCs are the better APCs but as with macrophage-derived foam cells no effect of foam-cell formation on OVA-17 induced proliferation (Figure 7C and 7D).

When we assessed the MHC-II processing capacities of foam cells, we again did not observe an effect of foam cell formation on D011.10 proliferation in response to ovalbumin uptake (Figure 8A and 8B) or OVA-IC uptake (Figure 8C and 8D) as determined by IL-2 production.

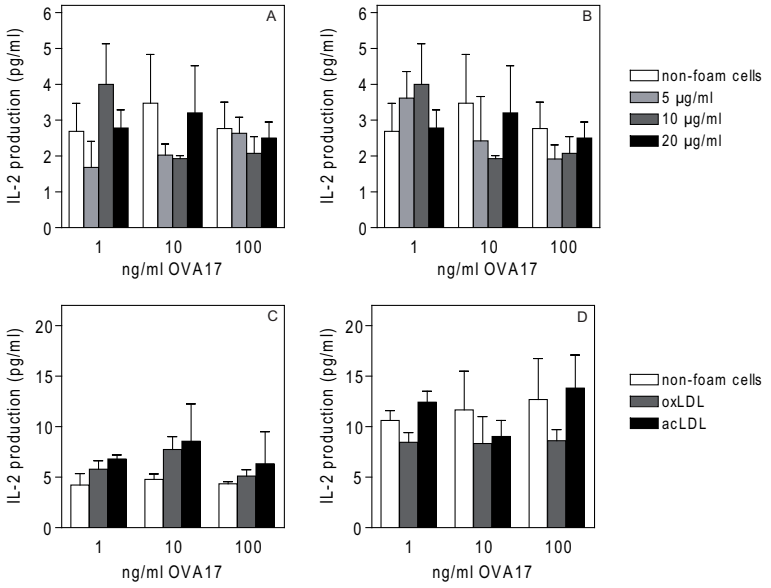

Figure 7: Foam cell fromation did not alter MHC-II antigen presentation
Bone-marrow derived MΦ were incubated for 24 hours with various concentrations of acLDL (A) or oxLDL (B). Also, we cultured MΦ (C) and DCs (D) in the presence of oxLDL (10 µg/ml) or acLDL (20 µg/ml). After foam cell formation, various concentrations of OVA-17 were added and MHC-II specific antigen processing was determined using D011.10 cells. Antigen-specific proliferation was not affected by foam cell formation in both MΦ and DCs.

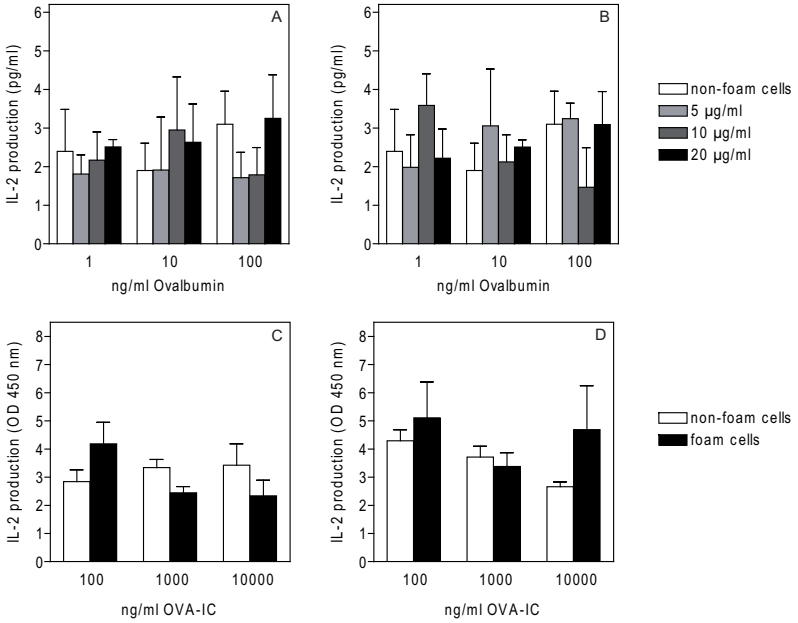

Figure 8: MHC-II processing was also not changed after foam cell formation
Bone-marrow derived MΦ were incubated for 24 hours with various concentrations of acLDL (A) or oxLDL (B) before incubation with ovalbumin. MHC-II processing was also determined with OVA-IC in MΦ and DCs after foam cell formation (C and D, respectively). MHC-II processing was not affected by foam cell formation in both MΦ and DCs.

These results were confirmed by using T cells derived from OTII mice. CSFE labeled T cells from OTII mice were stimulated with APCs that were incubated with OVA-17 or OVA-IC. Again, we did not observe an effect of foam-cell formation on MHC-II antigen presentation and processing. However, these data again confirmed that DCs are better APCs then MΦ with respect to MHC-II antigen presentation (Figure 9A and B, respectively) and processing in MHC-II (Figure 9C and D, respectively).

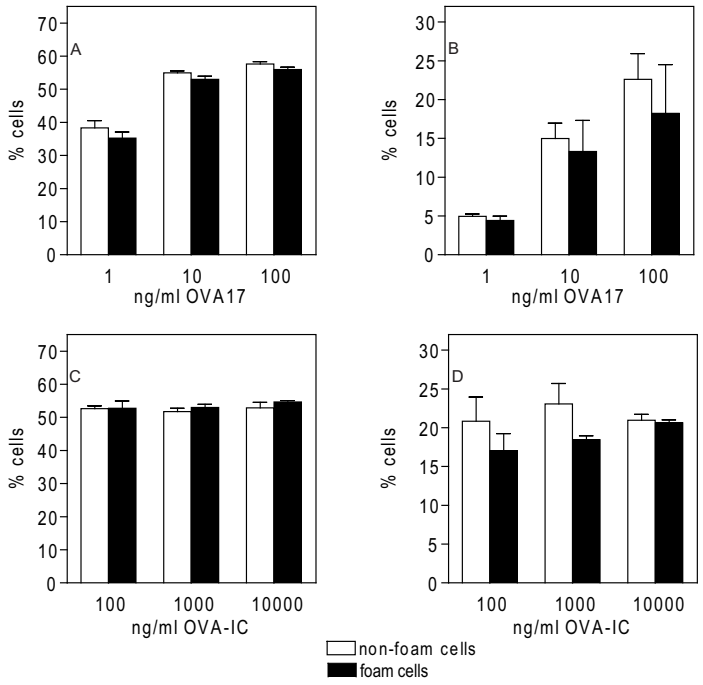

Figure 9: Proliferation of OTII cells was not affected by foam cell formation
T cells were isolated from OTII mice and labeled with CSFE. Next, OTII cells were cultured in the presence of MΦ or DCs that have been incubated with OVA-17 (A and B, respectively) or OVA-IC (C and D, respectively) and proliferation was measured using flow cytometry. The percentage of cells within the MFI region for 5 cell cycles was analyzed.

Hypercholesteremia increases CD11c expression in macrophages *in vivo*
Since foam cell formation affected the antigen presentation capacity of macrophages and their CD11c expression *in vitro*, we next assessed the effect of hyperlipidemia on the mΦ phenotype. Therefore, we compared the expression of DCs markers within the macrophage populations in LDLr$^{-/-}$ mice that were fed a western-type diet for 10 weeks to mice that were fed a chow diet. Western type-diet feeding increased the percentage of CD11c^{+}CD11b^{+} cells in blood, spleen and liver (Figure 10A). This increase was mainly due to an increase of CD11c^{high}CD11b^{+} cells (Figure 10B). Also the MHC-I expression was increased within CD11b^{+} cells after diet feeding (Figure 10C). More interstingly, the maturation status of both DCs and MΦ was increased in blood, spleen and liver of hypercholesteremic mice as indicated by the increased percentage of CD86^{+} cells (Figure 10D and E).

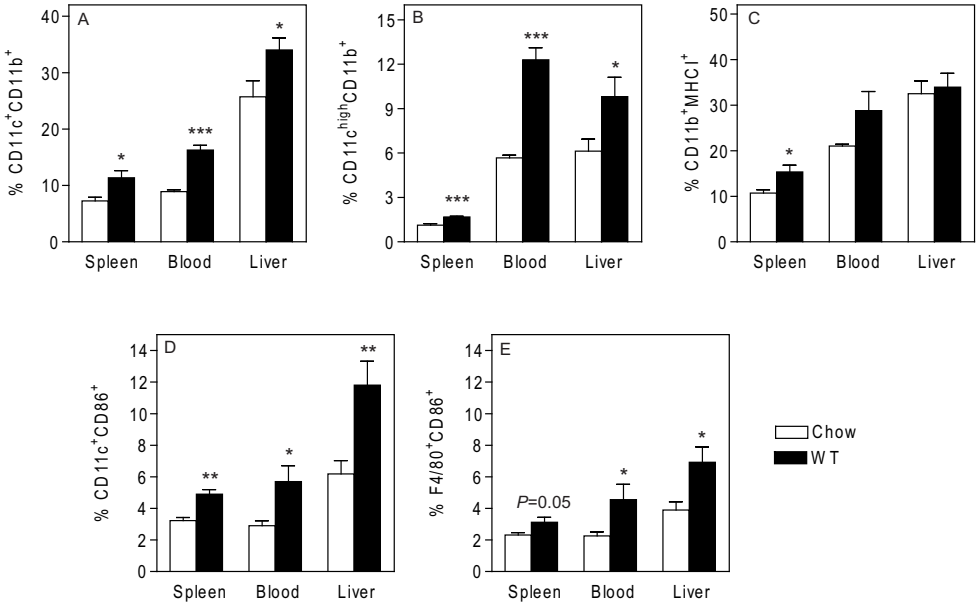

Figure 10: CD11c expression is enhanced in macrophages and APC are more activated under hypercholesteremic conditions
Percentage of $CD11c^{+}CD11b^{+}$ cells is increased in organs of $LDLr^{-/-}$ after western-type diet feeding of 10 weeks (A). This increase was mainly due to the increase of $CD11c^{high}$ cells within the MΦ population (B). Also MHC-I is upregulated in MΦ after diet feeding (C). Both DCs and MΦ are more activated after diet feeding as indicated by the higher % of CD86 double positive cells (D and E, respectively).

Discussion

Recent data suggest that foam cell formation has immunomodulatory properties affecting the capacity to present antigens by DCs and MΦ. The migration of DCs is impaired under hypercholesteremic conditions but on the other hand, recent studies show that CD11c expression is upregulated in MΦ upon foam cell formation. For instance, oxLDL induces the differentiation of the murine macrophage cell line RAW264.7 into dendritic-like cells with an increased expression of co-stimulatory molecules and MHC-II expression.[10] In addition, micro-array analysis of human monocyte derived macrophages isolated form blood also reveals a higher expression of CD11c and MHC-II after treatment with oxLDL.[9] Our present data on the effect of foam cell formation on antigen presentation by macrophages showed that the incubation of bone-marrow derived MΦ with oxLDL or acLDL increased CD11c expression to an expression level comparable to the level observed in DCs. More interestingly, the expression of MHC-I, MHC-II and costimulatory molecules was increased suggesting that foam cell formation may affect their antigen presenting capacities.

In order to test the antigen presenting capacity of the macrophage derived foam cells, we incubated foam cells with the MHC-I specific peptide SIINFEKL or the MHC-II-specific peptide OVA-17 and determined the antigen-specific proliferation using B3Z and D011.10 cells, respectively. We observed a significant increase in SIINFEKL induced proliferation of B3Z cells when we cultured bone-marrow derived MΦ with increasing concentrations of oxLDL or acLDL. DCs also demonstrated an enhanced MHC-I dependent proliferation upon foam cell formation. In addition, we also demonstrated that not only the direct presentation of peptides was enhanced, also uptake and subsequent processing of antigens was increased in MΦ-derived foam cells. This is in contrast to the effects seen in DC-derived foam cells. However, this may be explained by the fact that oxLDL uptake induces the maturation of DCs, which limits subsequent antigen uptake and presentation by the DCs. OxLDL is known to induce maturation but not to the same extent as LPS. During full maturation, DCs loose their capacity to phagocytose antigens. In our present study we showed an induction of CD86 and MHC-II in DC-derived foam cells, indicative of an induction of maturation while the expression of these markers remained low in MΦ. However, the uptake of fluorescently labeled ovalbumin was unaffected in DCs and MΦ suggesting that foam cell formation did not affect the uptake of the antigens but may affect the processing and subsequent antigen presentation.

In contrast to the enhanced MHC-I presentation by MΦ-derived foam cells, we did not observe any effects of foam cell formation on MHC-II presentation or processing as determined by proliferation assays using D011.10 cells or CSFE labeled OTII cells. This finding is in accordance with the study of Packard *et al.* who observe no effect of foam cell formation on isolated DCs on the MHC-II specific processing and presenting capacities of intact ovalbumin and ovalbumin-derived peptides by DCs. These effects are observed both *in vitro* after cholesterol loading with acLDL and *in vivo* in hypercholesteremic mice.[13]

Next we assessed the effect of hypercholesteremia on the MΦ population in LDLr$^{-/-}$ mice. We also observed *in vivo* an induction of a more DC-like phenotype of MΦ

in mice that are fed a high-cholesterol high-fat diet. The expression of CD11c on CD11b positive macrophages was increased in spleen, blood and liver. Moreover, both MΦ and DCs are more matured in mice being fed the western-type diet, although the macrophages and dendritic cells are not full foam cells under these conditions. These data are supported by the findings of Wu *et al.* who not only observes higher levels of CD11c on circulating mouse blood monocytes but also observes an increased granularity and increased expression of activation markers such as CD29.[11]

The observation of increased CD11c expression in MΦ in mice on a high fat diet may have major implications for atherosclerosis. Recently it has been shown that especially the firm arrest after rolling under shear flow conditions is enhanced in CD11c expressing cells.[11] In addition, patients with coronary artery disease have decreased numbers of both circulating plasmacytoid and myeloid dendritic cells which could be explained by an active recruitment of DCs into the lesions.[14] We saw an increase of CD11c expressing MΦ in blood suggesting more arrest to the endothelial wall and subsequent transmigration into the atherosclerotic plaques. Moreover, foam cell MΦ are abundantly present in early lesions. Our present data therefore suggests that the presence of foam cell MΦ with a DC-like phenotype can induce a local induction of a $CD8^+$ T cell response. Indeed, the inflammatory infiltrate of human plaques shows within the T cell population a slight predominance towards the $CD8^+$ phenotype.[15] In addition, the CD4/CD8 ratio in the plaque is lower than in the circulation which already indicates the shifted balance between $CD4^+$ and $CD8^+$ T cells.[15] Moreover, in the study of Rossmann *et al.* it is shown that the $CD8^+$ T derived from human carotid arteries cells have an increased IFN-γ secreting capacity compared to $CD4^+$ cells which consequently could activate resident T cells and MΦ.[15, 16] In addition, $CD8^+$ T cells have been found to play a predominant role in aneurysms formation and Kawasaki disease.[17, 18]

In conclusion, in the present study we show that foam-cell formation affects the antigen presentation and processing capacities of both MΦ and DCs. We showed for the first time that foam cell formation not only induces a DC-like phenotype in macrophages but it also enhances MHC-I antigen processing and presentation *in vitro*. Moreover, we verified our *in vitro* data by showing increased CD11c expression in the MΦ population and a more activated phenotype after western-type diet feeding. Taken together, our current findings indicate that foam cell formation may have major implications on the migration, activation and stimulating capacities of monocytes and consequently, in the initiation of atherosclerotic lesion formation. Furthermore our data suggests a possible role for $CD8^+$ T cell activation in early lesion development as *in vitro* MHC class I proliferation is increased in macrophage-derived foam cells.

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