The release of cytokines by macrophages is not affected by myelin ingestion
ABSTRACT

Macrophages play an important role in demyelination in Multiple Sclerosis (MS). Activated macrophages ingest myelin particles, thereby acquiring a foamy appearance. Foamy macrophages in MS lesions were described as being anti-inflammatory. Therefore, these cells might play a role in changing the inflammatory state of an active lesion. In this study we investigate the mechanism by which myelin uptake leads to skewing of macrophage activation status towards an anti-inflammatory phenotype. Macrophages were incubated with myelin leading to the development of foamy macrophages. Afterwards, the cells were stimulated with the TLR-4 ligand lipopolysaccharide (LPS) and cytokine production was determined. Foamy macrophages showed reduced cytokine production and appeared LPS insensitive. Next, we investigated the cytokine secretion during foam cell formation. Both tumor necrosis factor (TNF)-α and interleukin (IL)-10 were produced by macrophages upon myelin ingestion. When foamy macrophages were generated with other myelin preparations neither induction of cytokines upon myelin ingestion nor LPS insensitivity was observed. These results led us to investigate the factor responsible for the different outcomes between different myelin batches. It turned out to be LPS in one of the myelin preparations. We demonstrated that LPS contamination induced insensitivity to LPS in foamy macrophages. On the contrary, LPS-free myelin did not induce foamy macrophages to become insensitive to LPS. To conclude, myelin-laden macrophages were not LPS insensitive, indicating that they had not acquired an anti-inflammatory phenotype.
Demyelination is one of the hallmarks in Multiple Sclerosis (MS) pathogenesis. Cells of the macrophage lineage are major effector cells of this process since infiltrating macrophages as well as activated microglia break down and ingest particles of myelin sheaths, thereby displaying a ‘foamy’ phenotype. Furthermore, elimination of macrophages in experimental autoimmune encephalomyelitis, an animal model for MS, resulted in complete suppression of clinical signs, stressing the predominant role of macrophages in MS and presumably demyelination. Despite extensive research, the exact trigger for macrophage activation in MS remains elusive. Based on presence of macrophages, lymphocytes, nitric oxide (NO) and cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-2 and interferon (IFN)-γ active MS lesions are considered pro-inflammatory.

Macrophages have a marked heterogeneity and can be classified in different subtypes. Development of these subtypes depends on environmental cues. The most extreme subtypes are M1 or classically activated (CA) macrophages, and M2 or alternatively activated (AA) macrophages. CA macrophages secrete pro-inflammatory mediators, while AA macrophages are involved in wound healing and immune suppression.

Opposed to the pro-inflammatory phenotype that CA macrophages acquire, lipid-laden macrophages possess anti-inflammatory features. Lipids such as high-density lipoprotein (HDL) and its major protein component, Apolipoprotein (Apo) A-1 elicit anti-inflammatory effects by inhibiting the production of IL-1β and TNF-α, and inducing IL-10 release. In addition, the glycosphingolipid glucocerebroside accumulates in macrophages of Gaucher disease patients as a result of defective activity of the enzyme glucocerebrosidase. Glucocerebroside-laden macrophages or so-called Gaucher cells, revealed anti-inflammatory features.

Similar effects were seen examining the effects of myelin lipids on macrophages. Boven et al. observed that myelin-laden macrophages have anti-inflammatory properties, since foamy macrophages in MS-lesions expressed anti-inflammatory cytokines, such as transforming growth factor (TGF)-β, IL-10 and IL-4. In vitro, foamy macrophages released more IL-10 and less TNF-α after lipopolysaccharide (LPS) stimulation compared to non-foamy macrophages. Furthermore, they appeared to be insensitive to activation by LPS. From these studies it appears that lipid-laden macrophages generally obtain anti-inflammatory features based on expression and release of anti-inflammatory mediators.

The presence of anti-inflammatory cells in pro-inflammatory, active MS lesions is interesting, since it may restrict lesion expansion, promote repair and extinction of inflammation. Here we aimed to unravel the mechanism of induction of an LPS insensitive, anti-inflammatory phenotype in foamy macrophages. It appeared that foamy macrophages, generated by myelin ingestion, were LPS insensitive but different batches of myelin differed in their effect on LPS tolerance. These differences appeared due to TLR-4 activation by LPS, contaminating one of the myelin pools used in this study.
Chapter 5

MATERIALS AND METHODS

Preparation and labelling of myelin

Human myelin was prepared as described previously. Brain samples (obtained from the Dutch Brain Bank) were suspended in 0.32 M sucrose and homogenized. Brain homogenate was layered over 0.85 M sucrose and centrifuged (75,000 g, 30 min). The interface with myelin was collected, suspended in de-ionized water and centrifuged (75,000 g, 15 min) (final step repeated twice). Myelin concentrations were determined by BCA assay (Thermo Scientific Pierce, Rockford, USA), using a BSA (Roche, Almere, the Netherlands) standard curve.

For phagocytosis experiments, myelin was labelled by incubating with the lipophilic fluorescent dye 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine-perchlorate (DiI) (Sigma-Aldrich, Zwijndrecht, the Netherlands) for 30 min at 37°C. Myelin was washed with phosphate buffered saline (PBS) by centrifugation (14,000 rpm, 20 min) and stored at -20°C. All myelin batches were processed according to the same protocol.

Human macrophages

Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy donor buffy coats (Sanquin Blood Bank, Amsterdam, The Netherlands) using Ficoll (Lymphoprep™, Axis-Shield, Oslo, Norway) density gradient. Monocytes were isolated from the PBMCs by Percoll (GE Healthcare, Uppsala, Sweden) density centrifugation. 2x10^6 cells/ml monocytes were seeded in Teflon flasks (Nalgene, Roskilde, Denmark) in macrophage medium (RPMI 1640 (Invitrogen, Breda, the Netherlands), supplemented with 5% (v/v) human serum (BioWhittaker, Verviers, Belgium), and 1% (v/v) PSG (Lonza, Breda, the Netherlands), at 37°C, 5% CO₂. After 5-7 days of culturing, monocytes differentiated into macrophages. All experiments were repeated with at least three different donors.

Cell Culture experiments

Macrophages were seeded in 96-wells plates (5x10⁴ cells per well) for 24 h. Unbound cells were removed by washing. Macrophages were incubated with myelin (50 µg/ml), Pmx (20 or 40 µg/ml) (Sigma-Aldrich) or LPS (10 ng/ml) (Sigma-Aldrich) for 48 h, unless otherwise mentioned. After myelin incubation supernatant was removed and collected, cells were washed and stimulated with LPS (10 ng/ml) 16 h after LPS stimulation supernatants were collected. Cell viability was assessed using WST assay, by incubation with WST-1 reagent (Roche) for 3 h. Absorbance was measured at 450 nm on an ELISA-reader (Model 680 Microplate Reader, Bio-Rad Laboratories, Veenendaal, The Netherlands).

To determine the effect of myelin on LPS activity, macrophages were incubated with myelin and LPS together or LPS alone. Supernatant was collected after 48 h.
**Phagocytosis experiments**

After 48 h of incubation with Dil labelled myelin macrophages were harvested using lidocaine (4 mg/ml, Sigma-Aldrich). Lidocaine was removed by centrifugation (1500 rpm, 5 min) and cells were resuspended in PBS/BSA 0.5%. The percentage of myelin-positive cells was measured by flow cytometry (FACSCalibur™, Becton Dickinson, Erembodegem, Belgium) and analysed using manufacturers software (Cellquest Pro). Alternatively, for microscopy cells were fixed with 3.7% formaldehyde and analysed with fluorescence microscopy (Nikon Eclipse TE300, Nikon Instruments Inc., Melville, USA).

**Cytokine assay**

Cytokine production in macrophage supernatants was determined by Cytometric Bead Array (CBA, Human Inflammatory Cytokines Kit, BD Biosciences, Erembodegem, Belgium) and performed according to manufacturer’s descriptions. The fluorescent signals were detected by flow cytometry and analysed by BD CBA Software.

**LAL assay**

LPS contamination was determined using the Limulus Amebocyte Lysate (LAL) assay (GenScript Corporation, Piscataway, USA). Brief, samples and endotoxin standard solution were mixed with LAL and incubated for 45 min at 37°C. Stop solution and subsequently color-stabilizing reagents were added. The absorbance was measured at 540 nm, using an ELISA-reader.

**Activation state of foamy macrophages**

Macrophages were exposed to either 5000 U/ml recombinant human IFN-γ (U-Cytech, Utrecht, the Netherlands) and 10 ng/ml *Escherichia coli* LPS (026:B6) for induction of CA macrophages or 10 ng/ml IL-10 and 10 ng/ml IL-4 (both recombinant human cytokines obtained from Immunotools, Friesoythe, Germany) for preparation of AA macrophages. Foamy macrophages were generated by exposure to 50 µg/ml of myelin (batch Mp2 or My-1). As a control, macrophages were left untreated (unstimulated macrophages, US). After 48 h of stimulation, cells were harvested with lidocaine (4 mg/ml), washed with PBS and fixed in PBS-formaldehyde 1%. Cells were stained for CD40 (Serotec, Oxford, UK) and mannose receptor (MR, BD Biosciences, San Jose, USA) since CD40 is highly expressed on CA macrophages and MR on AA macrophages. Analysis was performed using flow cytometry.

**Statistical analysis**

Statistical evaluation was performed by SPSS 15.0.0 (Chicago, USA), using a one-way ANOVA with Bonferroni correction. P <0.05 was indicated as statistically significant.
RESULTS

Formation of foamy macrophages

First, we investigated whether foamy macrophages were formed. Macrophage morphology was assessed after incubating cells with Dil-labelled myelin, batch Mp2, for 48 h. Myelin particles appeared to be phagocytosed by macrophages and present in the cytoplasm. Macrophages displayed the typical foamy appearance (Fig. 1B). Compared to control macrophages, 90% of cells had taken up myelin (Fig. 1C, D) assessed using flow cytometry. As a result of these findings, macrophages were classified as foamy macrophages when cells were incubated with myelin for 48 h.

Foamy macrophages showed reduced TNF-α and IL-10 production after TLR-4 triggering by LPS

Next, we investigated whether foamy macrophages expressed anti-inflammatory characteristics after TLR-4 triggering by LPS, as was shown previously 14. Macrophages were incubated for 48 h with increasing doses of myelin (10, 25, 50, and 75 µg/ml) or medium alone. After 48 h cells were exposed to LPS. Compared to control macrophages, foamy macrophages secreted substantially lower TNF-α concentrations in a myelin dose dependent manner (Fig. 2). Control macrophages released around 57 ± 13 pg/ml IL-10 upon LPS stimulation, while IL-10 concentrations derived from foamy macrophages were undetectable (Fig. 2).

Figure 1: Microscopic evaluation and FACS analysis of macrophages and foamy macrophages. Macrophages were cultured with medium only (A, C) or 50 µg/ml myelin Dil (B, D) for 48 h. Myelin (red) was ingested by macrophages and present adjacent to the nucleus (B). FACS analysis showed that 90% of cells had phagocytosed myelin particles (D), compared to control macrophages (C). Scale bar 50 µm. Results are representative of three individual experiments.

Figure 2: Cytokine release of control and foamy macrophages after TLR-4 activation by LPS. Macrophages were cultured in the absence or presence of myelin for 48 h and stimulated with LPS for 16 h. In foamy macrophages the LPS induced TNF-α secretion was attenuated compared to control macrophages. 57 ± 13 pg/ml of IL-10 was secreted by control macrophages and undetectable amounts by foamy macrophages. Results of three separate experiments are presented as mean ± SEM. * P<0.05, ** P<0.005.
Cytokine production by foamy macrophages

*Myelin phagocytosis induced TNF-α and IL-10 production by macrophages*

In MS lesions, foamy macrophages expressed anti-inflammatory molecules and cytokines, and lacked expression of the pro-inflammatory cytokines TNF-α, IL-1β and IL12p40/70. We evaluated whether foamy macrophages possess a similar phenotype *in vitro* by determining the TNF-α and IL-10 production. Macrophages were incubated for 48 h in the absence or presence of increasing doses of myelin (10, 25, 50, and 75 µg/ml), and spontaneous cytokine production was measured. Both TNF-α and IL-10 were produced by foamy macrophages in a myelin dose-dependent manner (Fig. 3A), while control macrophages did not release any of these cytokines.

Next, we determined the time course of cytokine secretion during foam cell formation. Cells were cultured for 2, 6, 8, 24, and 48 h in the presence of myelin or medium. Control macrophages released undetectable amounts of TNF-α and IL-10 (Fig. 3B, open squares). Substantial TNF-α secretion started 4 h after myelin exposure and increased further until it reached a concentration of approximately 1400 ± 194 pg/ml 8 h after priming (Fig. 3B, closed squares). At later time points, TNF-α secretion declined and finally after 48 h a concentration of 100 pg/ml was reached. The time course for IL-10 secretion was similar to TNF-α: macrophages released about 97 pg/ml at its maximum at 8 h and reduced with time (Fig. 3B).

To assess whether the effect of myelin on macrophages is batch-dependent we repeated the experiments with two other myelin preparations (batch My-1 and My-2). The percentage of myelin positive macrophages after 48 h was similar compared to cells that were incubated with myelin batch Mp2 (data not shown), indicating that different myelin preparations did not lead to differences in phagocytosis. Exposure to both My-1 and My-2 did not induce TNF-α (Fig. 4) or IL-10 (data not shown) production by macrophages. Next, we investigated the
cytokine profile of macrophages that were exposed to myelin My-1 and My-2 for 48 h and subsequently stimulated with LPS. In contrast to the previous experiments using myelin batch Mp2 (Fig. 2), both My-1 and My-2 did not significantly in- or decrease TNF-α secretion after LPS stimulation (Fig. 4). IL-10 production by LPS-treated foamy macrophages was not detectable.

**Figure 4: TNF-α secretion of myelin treated macrophages, before and after TLR-4 activation.** Macrophages were cultured with My-1 and My-2 myelin or left untreated for 48 h and stimulated with LPS. TNF-α concentrations before and after LPS stimulation were determined. Myelin phagocytosis did not induce TNF-α release by macrophages (closed squares and diamonds). Control and foamy macrophages released high levels of TNF-α after LPS stimulation (open squares and diamonds). Representative results are presented as mean ± SEM.

*TNF-α attenuation caused by LPS contamination*

Since different myelin batches had distinct effects on macrophage functional phenotype, we assessed whether a specific factor in the myelin pool was responsible for the induction of LPS insensitivity in foamy macrophages when cells were exposed to myelin batch Mp2. Since it was shown that the endotoxin LPS could be a frequent contaminant in multiple reagents and LPS is known to induce LPS tolerance through TLR4 triggering, we focused on excluding the possibility of LPS contamination.

We showed LPS tolerance by exposing macrophages to LPS for 48 h and subsequently re-stimulated with a second LPS dose (10 ng/ml, Fig. 5). Primary LPS incubation induced TNF-α and IL-10 secretion (Fig. 5). After a second LPS stimulation, both TNF-α and IL-10 secretion were reduced. In previous experiments using myelin batch Mp2 comparable results were observed.

**Figure 5: TNF-α and IL-10 release of LPS treated macrophages before and after a second LPS stimulation.** Macrophages were cultured in the absence or presence of LPS for 48 h. Supernatant was removed, and cells were re-stimulated with LPS (10 ng/ml). Macrophages secreted TNF-α and IL-10 in an LPS dose dependent manner (open squares). Both TNF-α and IL-10 were reduced after a second stimulation with LPS (closed dots). Data are expressed as mean ± SEM. Figure is a representative of three independent experiments. * P<0.05, ** P<0.005.
To exclude LPS contamination being responsible for both LPS insensitivity (Fig. 2) of foamy macrophages and TNF-α production at 8 h after myelin (batch Mp2) ingestion (Fig. 3B), we added the LPS inhibitor Pmx to the test system. Pmx is a cationic polypeptide that binds to the lipid A region of LPS, and thereby abrogates LPS-induced responses. Macrophages were exposed to myelin, Pmx and/or LPS for 24 h. TNF-α secretion was highest in macrophages treated with LPS or Mp2 myelin, 2500 ± 463.1 resp. 2200 ± 171.9 pg/ml (Fig. 6A). Pmx itself did not have any effect on macrophage TNF-α production. Addition of 20 or 40 μg Pmx reduced TNF-α secretion induced by both LPS and myelin Mp2 (250 ± 50.0 pg/ml) substantially. The decrease of TNF-α secretion by a combination of myelin Mp2 and Pmx, indicates LPS contamination in the myelin pool. As expected, incubation with My-1 or My-2 myelin did not lead to TNF-α production. The WST assay demonstrated that Pmx did not have an effect on macrophage viability (Fig. 6B). In addition, myelin uptake was not inhibited by Pmx, as was analysed by microscopy (data not shown).

Figure 6: The effect of Pmx on TNF-α production by macrophages. A) Macrophages were cultured with LPS, myelin (batch Mp2, My-1, or My-2), Pmx (20 and 40 μg/ml), or a combination. After 24 h incubation, supernatant was harvested and TNF-α concentrations were measured. Both LPS and myelin Mp2 induced high TNF-α secretion, however My-1 and My-2 did not. Co-incubation of Pmx with myelin Mp2 or LPS decreased TNF-α secretion substantially. No significant differences were noticed between 20 and 40 μg/ml pmx. B) Pmx did not have any effect on macrophage viability. Data are expressed as mean ± SEM of four independent experiments. n.m. = not measured * P<0.05, ** P<0.005.

To further evaluate LPS contamination in myelin, we performed a LAL assay (table 1). High endotoxin concentrations were established in myelin batch Mp2 (3.78 ± 0.4 ng/μg myelin), while My-1 and My-2 were endotoxin-free (levels below detection limit). These results indicate that the effect of myelin Mp2 was indeed associated with LPS contamination.

Table 1: Endotoxin concentrations in different myelin preparations. Endotoxin contamination in different myelin preparations (Mp2, My-1, My-2) was assessed using a LAL assay. B.d.l. = below detection limit of 1 pg/ml. Represented is the mean ± SD.
Chapter 5

Noteworthy is that LPS concentrations in Mp2 myelin are much higher compared to the LPS concentration used for stimulation. The Mp2 myelin induced TNF-α secretion by macrophages is not significantly higher (Fig. 6). To determine the effect of myelin on LPS activity we incubated macrophages with a combination of LPS-free myelin together with LPS and LPS only. Figure 7 shows reduced TNF-α concentrations released by macrophages when cells were incubated with both LPS and myelin, compared to cells exposed to LPS alone.

Figure 7: TNF-α secretion of LPS treated macrophages in the presence of myelin. Macrophages were exposed to LPS in the presence or absence of LPS-free myelin (My-1) for 48 h. TNF-α concentrations in supernatants were determined and cells exposed to both myelin and LPS produced less TNF-α compared to cells exposed to LPS only. Data are expressed as mean ± SEM of three independent experiments.

Foamy macrophages do not seem to possess a distinct phenotype

To determine whether foamy macrophages exist in either a more classically or alternatively activated state, their phenotype was compared to CA and AA macrophages. CA macrophages expressed higher levels of CD40, while MR expression is increased in AA macrophages (Fig. 8). Foamy macrophages (generated with myelin batch Mp2 and My-1) did not express CD40 and MR to the extent of CA and AA macrophages (Fig. 8). The expression level of both markers was much lower compared to CA and AA macrophages, indicating that foamy macrophages were not skewed towards a CA or AA phenotype. Furthermore, foamy macrophages generated with My-1 expressed both markers almost twice as high compared to foam cells generated with Mp2.

Figure 8: Expression of CA and AA markers on foamy macrophages. The expression level of CD40 and MR was determined on unstimulated (US), CA, AA and foamy macrophages (generated using Mp2 or My-1) by flow cytometry. CD40 was highly expressed on CA macrophages and MR on AA macrophages, compared to US macrophages. Foamy macrophages did not express these markers to the level of CA and AA macrophages. Results are representative of 3 independent experiments. Data represented as mean ± SEM.

DISCUSSION

Macrophages are the major effector cells of MS demyelination. Activated macrophages phagocytose myelin particles, resulting in a foamy phenotype. The
aim of our study was to investigate the mechanism by which macrophages become tolerant to LPS stimulation after myelin phagocytosis and obtain anti-inflammatory properties, as described for foamy macrophages in MS lesions 14.

After TLR-4 activation by LPS, foamy macrophages developed an LPS insensitive phenotype, as described before 14. However, when macrophages were exposed to other myelin preparations these results could not be repeated. LPS contamination was responsible for the different outcomes between myelin batches.

A recent publication described similar results regarding LPS contamination 24. The authors re-investigated the anti-inflammatory effects of adiponectin and found the effects were caused by LPS present in adiponectin. Adiponectin avidly binds LPS 25. Furthermore, Erridge et al. 26 found that saturated fatty acids did not stimulate TLR signalling, as was hypothesized, but TLR activation was caused by LPS contamination of the fatty acids. Finally, in a number of commercial reagents and preparations LPS was present 20. These studies, including ours, show that LPS contamination can be a serious problem and can lead to false results.

Remarkably, we found considerably higher LPS concentrations in Mp2 myelin compared to the amount of LPS used for stimulation, although the response by macrophages, release of TNF-α, was equal (Fig. 6). Perhaps myelin itself is responsible for inhibiting the pro-inflammatory effect of LPS without affecting the activation status of macrophages. We tested this hypothesis by exposing macrophages to LPS, with or without LPS-free myelin. Cells exposed to both LPS and myelin showed lower TNF-α concentrations compared to LPS-treated cells. Foamy macrophages (generated with LPS-free myelin) release equal amounts of TNF-α after removing the myelin and exposure to LPS, compared to macrophages exposed to LPS (figure 4). Therefore it seems plausible that LPS can be masked by myelin. In 1981 it was published 27 that myelin basic protein interacts with the lipid A region of LPS, thereby modifying the biological activity of LPS.

Contradictory results were found concerning induction of pro-inflammatory mediators by myelin phagocytosis. Van der Laan et al. 28 found, in line with our experiments, that myelin phagocytosis induced TNF-α and NO production. From our study it is clear that the cytokine secretion we observed was related to LPS contamination, while their assay showed undetectable levels (lower than 50 pg/ml) of LPS in the myelin. These differences might be explained by the differences in species (human versus rat) and source of macrophages (monocyte derived versus peritoneal). Another report did not find any production of the pro-inflammatory cytokine IL-12p40 upon myelin ingestion in human macrophages 14. We showed that human foamy macrophages primed with LPS-free myelin released equal amounts of TNF-α and IL-10 compared to control macrophages, both before and after LPS stimulation. These results indicate that foamy macrophages formed with LPS free myelin did not acquire an anti-inflammatory phenotype. However, it was previously demonstrated that myelin-laden macrophages possess anti-inflammatory characteristics 14. One explanation for these different results is that myelin composition differs between donors. Some reports described variations between myelin lipid composition between MS patients and healthy donors 29,30. We only used myelin from healthy controls. Myelin is rich in lipids. The regulatory
properties of lipid components have been investigated. Hyka et al. showed that IL-1β and TNF-α production by monocytes activated by T-cells, was inhibited by Apo A-I. Activation of monocytes by other stimuli did not inhibit the cytokine release, suggesting that T-cell signalling is necessary for the inhibitory function of Apo A-I on cytokine secretion by monocytes. This may also be the case for foamy macrophages in MS lesions, since T-cells are present there. Instead of Apo A-1, lipid compounds of myelin such as cholesterol might elicit inhibition of inflammatory mediators. Furthermore, HDL can prevent inflammatory responses, e.g. by inhibiting the expression of CD11b on monocytes after PMA stimulation. These latter results indicate that monocytes/macrophages obtain anti-inflammatory properties after lipid ingestion. However, we described that myelin lipid ingestion did not skew macrophages towards a fully anti-inflammatory phenotype, in absence of T cells.

Finally, we determined the presence of markers representative for CA or AA macrophages on foamy macrophages. Neither foam cells generated with Mp2 nor My-1 myelin expressed CD40 and MR to the extent of CA and AA macrophages, indicating that foamy macrophages were not skewed towards an anti-inflammatory phenotype, as was also observed by cytokine secretion by foam cells.

Furthermore, it seems remarkable that CA macrophages, induced by IFN-γ and LPS, expressed high levels of CD40, while foam cells generated with myelin containing LPS, did not express CD40 to the extent of CA macrophages. Obviously IFN-γ is necessary for induction of a CA phenotype and upregulation of CD40 on CA macrophages.

In summary, due to LPS contamination in our myelin preparation, foamy macrophages became insensitive to subsequent LPS stimulation. However, foamy macrophages formed with LPS-free myelin, responded to LPS after TLR-4 activation, indicating that foamy macrophages did not have an LPS insensitive phenotype nor became fully anti-inflammatory.

REFERENCE LIST

Cytokine production by foamy macrophages


