Activation Down-Regulation of Microglial Immune Inflammatory T Cells Leads to Microglial Phagocytosis of Apoptotic

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Microglial Phagocytosis of Apoptotic Inflammatory T Cells Leads to Down-Regulation of Microglial Immune Activation

Tim Magnus, Andrew Chan, Oliver Grauer, Klaus V. Toyka, and Ralf Gold

Apoptotic cell death is an established mechanism to terminate an inflammatory response in rodent or human brains. Microglia, as the resident phagocyte, is a strong candidate for the clearance of apoptotic lymphocytes. Apoptosis was induced in cultured autologous thymocytes and in myelin basic protein (MBP)-specific, encephalitogenic T cells from Lewis rats by the addition of 0.1 μg/ml methylprednisolone. The amount of phagocytosis of apoptotic cells was assessed using an in vitro phagocytosis assay. Supernatants were collected to measure microglial cytokine secretion. The state of immune activation in microglia was investigated by a T cell proliferation assay and by flow cytometric analysis of microglial surface expression of immune molecules. Microglia ingested specifically apoptotic cells (apoptotic thymocytes as well as MBP-specific T cells) in contrast to nonapoptotic control cells ($p < 0.0001$). Subsequent secretion of the proinflammatory cytokines TNF-α and IL-12 was significantly decreased, while the secretion of IL-10 and TGF-β was not affected. Furthermore, ingestion of apoptotic cells led to increased microglial MHC class II expression without concomitant increase in MHC class I, costimulatory molecules, and ICAM expression. The Ag-specific activation of MBP-specific T cells in cocultures with microglia that had ingested apoptotic cells was significantly less than that of identical T cells that interacted with nonphagocytosing microglia. Together with negative results obtained in a trans-well system, this is in support of a cell contact-mediated effect. Microglia might play an important role in the clearance of apoptotic cells. The uptake of apoptotic cells by microglia is tolerogenic and results in a reduced proinflammatory cytokine production and a reduced activation of encephalitogenic T cells. This might help to restrict an autoimmune inflammation and minimize damage in the inflamed brain. The Journal of Immunology, 2001, 167: 5004–5010.

A poptosis is an established way to terminate an autoimmune T cell response in rodent or human brains (1, 2). In experimental autoimmune encephalomyelitis (EAE), an apoptosis rate of infiltrating T cells of up to 50% has been observed (1, 3), and in the only reported post mortem case of acute demyelinating encephalomyelitis, ~30% of the T cells were apoptotic (4).

Through apoptosis cells loose their ability to respond to receptor signals and deactivate their enzymes (5). In neutrophils undergoing apoptosis, the toxic contents are sealed by an intracellular protein membrane cross-linking (6–8).

If apoptotic cells are not cleared rapidly, they will go into secondary necrosis. Neutrophils and T cells can release significant amounts of toxic enzymes, which might be harmful to the surrounding tissue (9). If secondary necrosis is abundant, the interstitial pH may drop and other lysosomal enzymes might be activated as well (10). At the same time, protease inhibitors may be inactivated by oxidation in tissues with dying cells (11). Therefore, it seems rather important that apoptotic cells are cleared in a fast and safe way. We were able to show that microglia, as a principle immune cell of the CNS, is capable of selectively ingesting great amounts of apoptotic cells (12).

We investigated the hypothesis that the process of engulfing apoptotic T lymphocytes leads to a profound down-regulation of the proinflammatory potential of microglia. It has been shown that macrophages that have ingested apoptotic cells secrete less pro- and more anti-inflammatory cytokines (13–15). The release of Fas-ligand by macrophages that have encountered apoptotic cells induces apoptosis in bystander leukocytes (16).

In the current study, we observed that microglia, upon interaction with apoptotic cells, secreted lower amounts of proinflammatory cytokine with no change in anti-inflammatory cytokine secretion, and was less potent as an APC. The inhibition of Ag-specific, encephalitogenic T cell activation seemed to be mediated through cell contact rather than through soluble mediators.

Materials and Methods

Isolation of microglial cells

Rat microglia cells were isolated from primary mixed brain glial cell cultures using a modification of previously described methods (17, 18). In brief, microglial cultures were prepared from the brain of newborn Lewis rats (P0-P2; Charles River Breeding Laboratories, Sulzfeld, Germany), which were freed of their meninges and minced with scissors under a dissecting microscope (Wild, Heerbrugg, Switzerland). Then the mixed cell cultures were grown in microglia medium (basal medium Eagle’s supplemented with 10% FCS (Sigma-Aldrich, Deisenhofen, Germany), 50 U/ml penicillin, and 50 ng/ml streptomycin) at 37°C for 10–14 days. Afterward, microglia cells were isolated by shaking the culture flasks for 7 h and adherence to a FCS-coated culture flask (Primaria; Falcon, Franklin Lakes, NJ). After trypsinization, microglia cells were resuspended and seeded into 48-well plates (Corning Glass, Corning, NY). To check the purity in our microglia cell culture system, we took a small fraction and performed immunohistochromie with the mAbs ED1 (1:50; Serotec, Kidlington, U.K.) and glial fibrillar acidic protein (1:100, DAKO, Hamburg, Germany).
Germany) as described (18). Ninety-five percent or more of the cells were ED1 positive and <5% were glial fibrillary acidic protein positive.

**Preparation of autologous apoptotic thymocytes**

This method has been described in detail (12, 19). In brief, apoptosis in freshly prepared autologous thymocytes was induced by adding 0.1 μg/ml methylprednisolone (MP; Hoechst, Frankfurt am Main, Germany) and propidium iodide (PI) in a flow cytometric analysis. In this analysis, the amount of annexin V-positive thymocytes in the MP-treated group reached levels of 45–50% with a proportion of 5–9% PI-positive late apoptotic or necrotic cells. Nonapoptotic control thymocytes showed 4–10% annexin V positivity and 2–5% PI positivity. The proportion of viable cells as assessed by trypan blue exclusion was consistently >97%.

**Preparation of myelin basic protein (MBP)-specific T cells**

A MBP-specific T cell line MBP 13 was generated as described (20). This CD4-positive Lewis rat T cell line is specific for the dominant encephalitogenic MBP epitope spanning amino acids (68–88). Apoptosis was induced with 0.1 μg/ml MP in the absence of IL-2 for 3 h at 37°C/5% CO2. The difference between apoptosis and necrosis was detected according to the same protocol as for thymocytes. The percentage of annexin V-positive in PI-negative gated cells was 30–35% with PI positivity of 5–8% of the cells. For the cells used as negative controls, annexin V positivity was 12–16% and PI positivity was 3–7%. The amount of viable cells stained with trypan blue was >97%.

Because apoptotic cells in vitro eventually proceed into secondary necrosis and the uptake of cells dying by apoptosis may be mediated by mechanisms other than the phagocytosis of necrotic cells (9, 14, 21), the modes of induction of apoptosis were modified to achieve a high level of apoptotic target cells with only a small proportion of secondary necrotic cells. This was of particular importance for the permanent, encephalitogenic T cell lines that were prone to enter a state of necrosis within a time frame of ~6–10 h. Viability of the different apoptotic cell populations used as phagocytic targets measured by trypan blue exclusion exceeded 97% in our study, a rate that corresponds to other well-established systems of phagocytosis of apoptotic cells (22, 23). In parallel experiments, their functional encephalitogenic activity was shown by using aliquots of the same cell preparation in animal models, which demonstrated clinically mild to severe adoptive transfer EAE.

**In vitro phagocytosis assay**

The assay that we used for our experiments is a modified version of a readout system that has been used on many different cell types (12, 18, 19, 23–27) and was adjusted to use microglia cells (12). In brief, 3 × 104 microglia cells per well were cultured overnight. The optimal number of microglia cells and thymocytes as well as MBP-specific T cells had been titrated previously (12). After a washing step with serum-free medium, 10 × 10^6/500 μl of apoptotic or nonapoptotic thymocytes as controls were added, and cells interacted for 2 h. The interaction was interrupted by washing with cold PBS. Following trypsinization, we performed cytocentrifuge preparation of the microglia cells. The cytocentrifuge preparation were stained with May-Giemsa (Merck, Darmstadt, Germany). To determine the amount of phagocytosing microglia and the number of ingested thymocytes, microglia cells, microglia cells were counted under light microscopy by an observer who was not aware of the cells added to the microglia. A minimum of 500 microglia cells per slide were counted.

**Cytokine secretion**

To analyze cytokine production, 3 × 10^3/well microglia cells were incubated overnight. After a washing step with serum-free medium, 10^6 apoptotic or nonapoptotic cells or no cells were added to each well of microglia cells in a volume of 500 μl for 2 h and were then carefully washed with 37°C warm medium to remove nonadherent cells. To ensure similar amounts of cytokine-producing microglia cells, the number of cells was checked by light microscopy. Microglia was stimulated with LPS to increase cytokine secretion because in pilot experiments, unstimulated microglia showed a rather low cytokine production. LPS (10 ng/ml) increased cytokine production to a maximal level, which declined due to the toxic effect after the administration of ultrahigh doses of LPS (1–10 μg/ml). In our experiments we used 10 ng/ml LPS. As a control, the supernatants of apoptotic and nonapoptotic cells after LPS stimulation were also analyzed, showing negligible amounts of cytokine production. Supernatants were collected at given time points, usually at 24 h, centrifuged to remove particulate debris, and stored in aliquots at ~70°C. Cytokine concentration was determined from the supernatants by ELISA, using immunoassay manufactured by R&D Systems (Minneapolis, MN). The cytokines analyzed were TNF-α, IFN-γ, IL-12, IL-10, and TGF-β1. Assays were performed according to the manufacturer’s instruction provided with each kit. ODs were detected on a microplate autoreader at 450 nm wavelength.

**T cell proliferation and activation studies**

In our system, unstimulated microglia, as the only APCs, did not lead to significant T cell proliferation. When adding IFN-γ (100 U/ml) to stimulate microglia APC functions, modest amounts of T cell proliferation could be observed. The effect, however, was much more pronounced when professional APCs had been added (28).

Microglia cells (10^5 per well) were seeded into 96-well flat-bottom microtiter plates, incubated for 24 h, and then allowed to interact with 3 × 10^5 apoptotic, nonapoptotic thymocytes or no cells for 2 h. The cells were then carefully washed twice with 37°C warm medium and irradiated (30 Gy). Resting MBP-specific T cells from the MBP-13 cell line at a concentration of 4 × 10^5 per well and 10^5 irradiated thymocytes were added to the microglia cells. Thymocytes were irradiated to prevent proliferation. In a latter subset of experiments, these thymocytes and the MBP-specific T cells were separated from the microglia cells after 4 h and were transferred to a different 96-well flat-bottom microtiter plate. To stimulate T cell proliferation, MBP or ConA at concentrations of 10 or 2.5 μg/ml, respectively, were used. The negative control was performed without Ag. The optimal number of microglia cells and the optimal amount of mitogen for the maximal T cell proliferation was titrated beforehand. Triplicate cultures were maintained at 37°C in a humidified atmosphere with 5% CO2. Cultures were pulsed with 0.2 μCi [3H]thymidine per well for 16 h and were harvested at indicated time points. The cells were collected on glass fiber filter paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finland). The radioactivity associated with the dried paper by a Betaplate 96-well harvester (Pharmacia-LKB, Turku, Finl...
IL-2-exposed T cells reached only 62.3 ± 11.5% (mean ± SD) of the phagocytosis rate of T cell growth factor-deprived, MP-treated apoptotic T cells (p < 0.0001). In these experiments, 36.2 ± 5% (range of 28.2–43.6%) of the total microglia were capable of phagocytosing cortisone-treated, apoptotic MBP-13 T cells.

**Proinflammatory cytokine secretion by microglia**

To determine whether the uptake of apoptotic cells by microglia would influence other microglial functions, cytokine secretion was analyzed. For this purpose, MP-treated apoptotic thymocytes, untreated thymocytes, or no thymocytes were added to microglia cells. The interaction was stopped after 2 h by the removal of the added thymocytes to avoid an influence by secondary necrotic cells.

When analyzing proinflammatory cytokines, we found a clear down-regulation of proinflammatory Th1-type cytokines. TNF-α production by microglia cells that had ingested apoptotic thymocytes decreased significantly when compared with microglia cells that had interacted with nonapoptotic thymocytes (p = 0.03). The same reduction of TNF-α production was found in comparison with TNF-α secretion of pure microglia cell cultures from the same preparation (p = 0.03; Fig. 2A).

In time kinetic experiments, TNF-α secretion by microglia increased exponentially until ~4 h after LPS stimulation, yet TNF-α release was clearly decreased after phagocytosis (Fig. 2B). A significant decrease was also observed for IL-12 production (Fig. 2C). The rat-specific ELISA kit detected the active, heterodimeric p70 form of IL-12 consisting of the p35 and p40 chain, as well as the inactive p40 subunit.

Very similar results could be obtained for microglia ingesting apoptotic, MBP-specific, and encephalitogenic T cells (data not shown). All tests were done with negative controls consisting of apoptotic or nonapoptotic cells alone where the cytokine concentrations in the supernatants would be measured 24 h after LPS stimulation. No relevant cytokine secretion was detected in these negative controls. A very similar modulation of microglial TNF-α secretion was found after apoptosis had been induced in thymocytes by irradiation instead of glucocorticoids.

**Anti-inflammatory cytokine secretion by microglia**

In contrast to the proinflammatory cytokines, the anti-inflammatory Th2-type cytokine production did not change after interaction with apoptotic/nonapoptotic cells (Fig. 3, A and B).

There was no significant difference in microglial IL-10 secretion whether apoptotic or nonapoptotic cells had been added before the analyses (p = 0.62). However, there was a clear increase in IL-10 production through the addition of thymocytes (Fig. 3A). The maximum of microglial IL-10 secretion occurred around 24 h, which was much later than the maximum of TNF-α secretion (data not shown).

As another Th2-type cytokine, we analyzed microglial TGF-β1 production. It is known that microglia can secrete fairly high concentrations of TGF-β (30). This was also true in our system, but there was no additional secretion, even after the addition of apoptotic cells to the microglia cell cultures.
data is from three independent experiments, each performed in triplicates. No influence on microglial TGF-β production could be detected after the addition of apoptotic cells in distinction from pure microglia cultures or cultures with nonapoptotic thymocytes. The experimental conditions are the same as in Fig. 2, and data is from three independent experiments, each performed in triplicates.

**Ag-specific T cell proliferation**

Because microglia cells themselves are weak stimulators of T cell proliferation (31), we added thymocytes as professional APCs to our cultures similar to previous studies on astrocytes (32).

Microglia that had ingested apoptotic thymocytes before interaction with MBP-specific T cells clearly reduced T cell proliferation relative to cultures where microglia had interacted with nonapoptotic or no thymocytes (Fig. 4A). For stimulation with MBP, this was significant at a level of $p = 0.03$ (vs cultures with non-apoptotic cells) and $p = 0.005$ (vs control cultures). With ConA stimulation (Fig. 4A), a very similar effect could be observed. T cell proliferation was decreased after addition of apoptotic cells in contrast to the addition of nonapoptotic cells or pure microglia cell cultures ($p = 0.02$ and $p = 0.002$, respectively).

The proliferative response differed in their time course depending on the length of incubation (Fig. 5B). Although the effect of a decreased T cell proliferation by phagocytosing microglia was visible until 48 h, it subsided after 72 h where a general inhibition of T cell proliferation by the addition of microglia cells was observed even though nonapoptotic and apoptotic cells in the microglia cultures were washed off before the addition of T cells, we wished to rule out an unspecific effect by these cells on the T cell proliferation rate. Therefore, we used nonapoptotic and apoptotic cells without microglia cells in cocultures with T cells as negative controls in every experiment. No influence of T cell proliferation could be detected. Irradiated microglia cells would not proliferate either.

To further rule out an unspecific effect due to phagocytosis of apoptotic thymocyte APCs by microglia in control cultures, APCs and MBP-specific T cells were separated from the microglia cells after 4 h of coculturing. Until this time point, thymocyte apoptosis was still low (33). After 24 h, MBP-specific T cell proliferation was reduced in cocultures where they had been incubated with microglia cells that had encountered apoptotic thymocytes by 75.5% or 69.4% when compared with microglia cells that were allowed to interact with nonapoptotic cells or microglia cells alone, respectively ($p = 0.002$ or $p < 0.05$, respectively).

To answer the question regarding whether the reduced T cell proliferation upon cocultures with microglia cells and professional APCs was due to soluble factors secreted by microglia that had ingested apoptotic cells or mediated by direct cellular contact, we conducted experiments where microglia cells were separated from T cells and APCs by a permeable membrane of a trans-well system. On day 2 or 3, T cells and APCs were transferred to microtiter plates, labeled, and harvested 1 day later. No inhibition of T cell proliferation was found. T cell proliferation was 9447 cpm ± 601 (mean ± SD) for pure microglia cultures, 8407 cpm ± 413 in cultures where microglia cells had interacted with nonapoptotic cells, and 9875 cpm ± 83 in cultures where microglia cells had ingested apoptotic cells ($p = 0.45$ and $p = 0.67$, respectively).

**Microglial expression of surface molecules**

To detect microglial surface expression, IFN-γ-stimulated microglia cells were analyzed by immunoflow cytometry. Through stimulation with IFN-γ, an elevation in MHC class II, ICAM, and B7-2

![FIGURE 3](image-url)  Microglial IL-10 and TGF-β production after interaction with apoptotic cells. A, IL-10 secretion by microglia cells did not change significantly when apoptotic cells (■) were added in comparison with non-apoptotic cells (□). In contrast, an increase in IL-10 production could be noticed with the addition of cells (apoptotic or nonapoptotic) to the microglia cells. The experimental conditions are the same as in Fig. 2, and data is from three independent experiments, each performed in triplicates. B, No influence on microglial TGF-β production could be detected after the addition of apoptotic cells (■) in distinction from pure microglia cultures (□) or cultures with nonapoptotic thymocytes (□). The experimental conditions are the same as in Fig. 2, and data is from three independent experiments, each performed in triplicates.

![FIGURE 4](image-url)  T cell proliferation after interaction with microglia and time dependence. A. The data on the graph represents cocultures of MBP-specific T cells, microglia cells, and professional APCs. Microglia cells were allowed to interact with apoptotic or nonapoptotic thymocytes for 2 h. After the thymocytes had been removed by washing, freshly prepared thymocytes as APCs, MBP-specific T cells, and Ag were added. Cells were labeled after 24 h and proliferation was measured. T cells counts declined after stimulation with MBP and ConA in cultures where microglia cells had engulfed apoptotic cells (+) significantly compared with microglia cultures with no additional cells (left bars) and nonapoptotic cells (–), respectively. The difference between the T cell proliferation rate in cultures with microglia cells (left bars) and microglia cells with so called nonapoptotic cells (–) was most likely caused by the fact that even in the nonapoptotic cell population a certain proportion of cells were apoptotic. Yet this difference was not statistically significant ($p = 0.12$). B, A decreased T cell proliferation by the addition of apoptotic cells to the microglia cells (□) could be found in time kinetic studies using MBP as a specific stimulation until 48 h. After 72 h, a general inhibition of T cell proliferation under all three culture conditions could be observed. The experimental conditions are the same as in A.

![Diagram](image-url)
expression could be observed. B7-1 expression could not be detected on our microglia cells. After an addition of apoptotic thy-
mocytes to the microglia cells, microglia up-regulated MHC class II expression when compared with nonphagocytosing microglia. In contrast, ICAM and also MHC class I expression was not affected by the ingestion of apoptotic cells. There was only a small amount of B7-2 expression, but it disappeared after the uptake of apoptotic cells (Fig. 5).

Discussion

In the present study, we have shown that rat microglia cannot only ingest apoptotic cells, but we have also shown that this process significantly alters microglial state of immune activation, thus rendering them tolerogenic. We found a significant decrease of proinflammatory cytokine secretion by microglia after they had phagocytosed apoptotic cells, whereas anti-
inflammatory cytokine production remained unaffected. This argues against a general down-regulation of cytokine production as an unspecific effect of microglial phagocytosis of apoptotic lymphocytes.

Furthermore, we demonstrated that MBP-specific T cell proliferation is markedly reduced in cocultures of microglia that had engulfed apoptotic cells and more so if professional APCs had been added. This experimental setup was chosen because micro-
glia cells alone did not show enough stimulatory capacity. This is well in line with the results from other groups that demonstrate that microglia from different sources is a relatively weak stimulator of T cell proliferation (31, 34–36). The use of irradiated thymic cells in in vitro assays as APCs for CNS-autoantigen specific T cells is well established and has been widely used in different model sys-
tems (20, 28, 37, 38). In our trans-well experiments, we did not see any influence on T cell proliferation, indicating that the inhibitory signals may be mediated through cell-cell contact rather than through soluble substances, even though unstable mediators (e.g. NO) cannot be excluded.

For complete T cell activation, APCs have to express certain costimulatory molecules. In vitro and in situ studies have demonstra-
ted that microglia cells are able to express costimulatory mol-
ecules (39–41). The findings of low expression of B7-2 with a lack of B7-1 expression (42) might depend on the cytokines used to stimulate microglia cells. IFN-γ seems to increase only the mRNA expression of B7-2 (43, 44). This corresponds to the findings in our study.

Regarding surface molecule expression, we found an increase in microglial MHC class II expression, but there was no influence on MHC class I and ICAM-1 expression. The increase in MHC class II expression might mediate the contact to the MBP-specific T cell, but overall leads to an inhibition of T cell proliferation. This could be due to a reduction of costimulatory molecules, as shown in this study.

Similar findings have been reported with regard to macrophages. The engulfment of apoptotic eosinophils by human macrophages failed to stimulate the release of the proinflammatory eicosanoid thromboxane and of the proinflammatory cytokine GM-CSF (14). Additional experiments indicated that macrophages also produce lesser amounts of TNF-α, IL-1β, and IL-12(13) after phagocytosis of apoptotic cells (15). Macrophages secrete more anti-
inflammatory cytokines in response to phagocytosis of apoptotic cells, including IL-10 (15), TGF-β1, prostaglandin E2, and plate-
let-activating factor (45). These effects could also be triggered by the binding of a newly described phosphatidylserine receptor (46).

In our system, we were not able to demonstrate an increase of anti-inflammatory cytokines, although our analyses were performed in the presence of serum.

Dendritic cells (DCs) are also capable of ingesting apoptotic cells (47, 48). In their immature state, DCs can phagocytose apo-
ptotic cells or necrotic cells, but only the uptake of necrotic cells leads to maturation. Interestingly, it seems that with additional stimulation, DCs can cross-present Ag from apoptotic cells to cy-
totoxic T cells, a feature that is not found in macrophages (49).

MBP-specific T cells and their interaction with microglia play an important role in diseases such as multiple sclerosis and EAE. Microglia cells are able to process and present native MBP (50) to MBP-specific Th1 and Th2 cells (51), and this seems to result in a proinflammatory response (52, 53). Furthermore, microglia can be stimulated to express MHC class II molecules and, therefore, is clearly able to interact with T cells (54, 55). For autoimmune diseases such as multiple sclerosis, MHC class II Ag expression was found on microglia in association with multiple sclerosis lesions (56), indicating a close T cell mi-
croglia interaction within these inflammatory lesions. Moreover, it seems that during the time course of chronic relapsing EAE in Lewis rats, the number of MHC class II-positive microglia in-
creases (57).

To what extent microglia and T cell interaction occurs in situ is still being debated, but the interaction between microglia and T cells is likely of functional relevance (58). Our findings of a func-
tional importance of the microglia T cell dialogue is supported by the observations of other research groups. For example, the Ag-
specific interaction of isolated microglia taken from animals with graft vs host disease with T cells led to proinflammatory cytokine production from a MBP-specific CD4 T cell line, yet there was no secretion of IL-2 or proliferation. After the encounter, T cells die by apoptosis (31). The same group of authors showed that there is substantial microglia activation by infiltrating T cells in graft vs host disease (59). The capacity of microglia to stimulate Th1 and Th2 cells depended mainly on their MHC class II expression (32). It seems that the amount of T cell proliferation and shaping of the cytokine repertoire depends on the activation state of the microglia cell (34–36, 42).
It is generally thought that microglial T cell activation helps to mediate autoimmune inflammation in the CNS. The different immune regulatory pathways of microglia cells may indicate a privileged reaction pattern in the CNS and may support the immune-privileged status of the brain (1). This may help to limit an incipient inflammatory lesion brought about by invading activated T cells. Once T cells are driven into apoptosis and phagocytosed, the concomitant local inflammation could be stopped by the mechanisms described in the present study in vitro.

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