

Impact of IVIg on the interaction between activated T cells and microglia

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When human microglia are co-cultured with activated human T lymphocytes, several cytokines become up-regulated in significant quantities. This condition can also occur at sites of inflammation in autoimmune inflammatory diseases of the central nervous system (CNS), including multiple sclerosis (MS), where T cells infiltrate the brain tissue and come in proximity to microglia. Therefore, T cell-microglia interaction is a potential avenue of drug therapy to decrease neuroinflammation. An immunomodulator used in autoimmune disorders is intravenous immunoglobulins (IVIg). The mechanisms of IVIg activity in diseases such as MS remain unclear. Here, we report that the application of IVIg to activated T cells leads to their decreased ability to engage microglia. As a result of IVIg treatment of T cells, there were reduced levels of tumor necrosis factor- α and interleukin-10 in T cell-microglia co-culture. Our results add to the understanding of how IVIg may affect inflammation of the CNS. [Neurol Res 2006; 28: 270–274]

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INTRODUCTION

The interaction between T lymphocytes and microglia, central nervous system (CNS) resident innate immune cells, is relevant to a number of autoimmune diseases, including multiple sclerosis (MS) where T cells infiltrate the CNS. When activated T cells come in close proximity to microglia, the latter becomes activated leading to the development of enlarged cell processes¹ and at least *in vitro*, the secretion of inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, IL-10 and IL-12^{2–4}. *In vivo*, T cells clustering around microglia has been observed in animal models of graft-versus-host disease⁵ and facial nerve resection⁶, and in adoptive T cell transfer experiments⁷.

The activation of microglia is believed to induce and/or maintain an inflammatory response within the CNS, as it involves increased capacity to present antigens to T cells and has impact on the recruitment, proliferation, differentiation and survival of infiltrating immune cells and endogenous neural cells^{8,9}. For example, it is known that activated microglia can induce neuronal death in cell culture¹⁰. The overproduction of certain pro-inflammatory cytokines by microglia and other immune subsets has been shown to enhance CNS pathology in different neurological diseases⁹. Therefore, treatments that normalize levels of cytokines such as TNF- α may decrease tissue injury in autoimmune diseases.

Interferon- β and glatiramer acetate, which are established treatments in MS, have been shown to decrease the secretion of specific cytokines, including TNF- α , IL-1 β , IL-6 and IL-12, when applied to activated T cells before their co-culture with microglia^{2,3}. These medications may not enter themselves into the CNS but their effect on T cells in the periphery of patients can alter the subsequent effect of T lymphocytes on CNS cells.

Here, we report that pre-treatment of activated human T cells with intravenous immunoglobulins (IVIg), another treatment used in MS, before their co-culture with microglia decreases TNF- α and IL-10 levels that are generated subsequent to the co-culture. Our findings have relevance to how immunomodulators used in MS affect the disease process.

METHODS

Isolation and treatment of T cells

Peripheral blood mononuclear cells (PBMCs) were obtained from blood of healthy volunteers by Ficoll Hypaque centrifugation as previously described^{3,11} and cultured in AIM-V medium (Gibco/BRL, Burlington, Ont., Canada). T cells were activated using an anti-CD3 antibody (OKT3) at a concentration of 1 μ g/ml. Flasks were kept lying flat and then stood up after 3 hours of culture in order to remove monocytes, which adhered to the plastic. After 3 days of anti-CD3 treatment, floating cells were collected and 100,000 cells in 100 μ l fresh AIM-V were plated onto 10,000 human fetal microglia per well of 96 well plate. Flow cytometry analyses of floating cells that were collected after 3 days of initiation of anti-CD3 treatment show CD3+ T cells to

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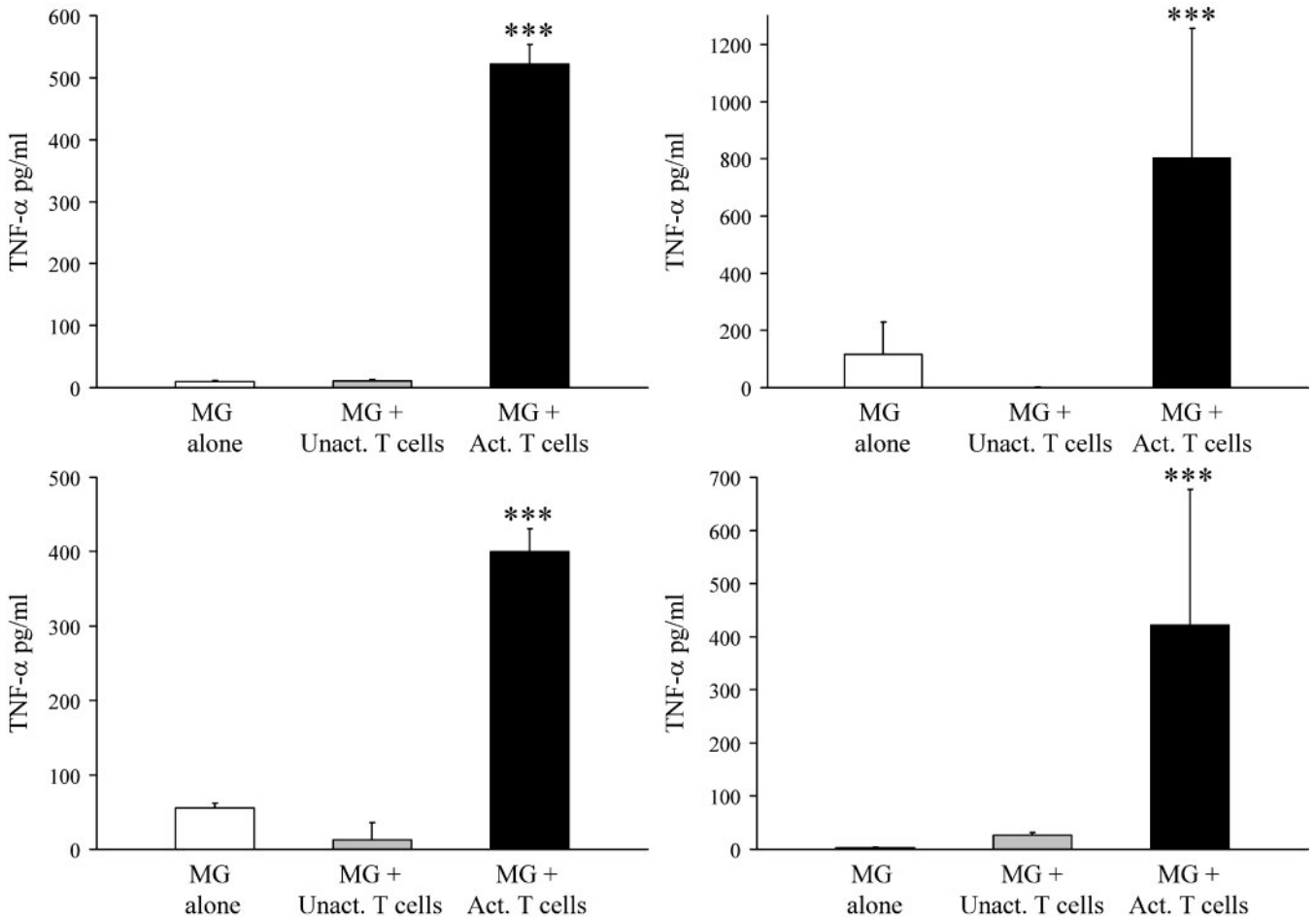


Figure 1: The interaction of activated T cells and microglia results in the significant generation of TNF- α . Displayed are four different experiments involving separate series of fetal human microglia with human T cells. In each case, microglia (MG) or microglia + unactivated (unact.) T cells produce low amounts of TNF- α into the culture medium. The interaction of microglia with activated (act.) T cells, however, resulted in significant increase in the level of TNF- α . *** $p < 0.001$ [one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons] compared with microglia alone or microglia + unactivated T cells. Values are given as mean \pm SD of triplicate cultures

constitute over 90% of the total cell population (unpublished observations). These are therefore referred to as activated T cells.

In cases where unactivated T cells were used, these were subjected to the above protocol except that no anti-CD3 treatment was applied.

For IVIg-pre-treatment of T cells IVIg (Gamunex, Talecris, Research Triangle Park, NC, USA) was added at different concentrations (0.1, 0.5, 1 and 5 mg/ml) 3 hours after the initiation of anti-CD3 treatment. Cells were left in culture for 72 hours and then centrifuged, washed, counted and 100,000 cells were plated onto 10,000 human fetal microglia/well.

Human fetal microglia cultures

Microglia cultures were obtained from brain tissues of human fetuses of 16–20 weeks fetal age following therapeutic abortion according to guidelines approved by local institutional ethics committees. Highly enriched microglia (>95% purity) were isolated as described previously⁴. Ten thousand microglia cells were plated onto each well of a 96 well plate (Nunc).

Microglia were cocultured with T cells for 24 hours 2–4 days after. Supernatants were collected and frozen at -70°C and later thawed for enzyme linked immunosorbent assay (ELISA).

ELISA

ELISAs for TNF- α and IL-10 were performed using commercially available kits from Biosource (Biosource International Inc., CA, USA) following the manufacturer's instructions.

RESULTS

Unactivated and activated T lymphocytes from healthy adult human donors were co-cultured with microglia derived from fetal human brains. After 24 hours, cell conditioned media were collected and submitted to ELISA for TNF- α . In sparsely seeded (10,000 cells/well) microglia cultures, levels of TNF- α were barely detected. When the microglia cells were incubated with unactivated T cells, there was no further increase in TNF- α levels (Figure 1). However, when microglia were co-cultured with activated T cells, there was a

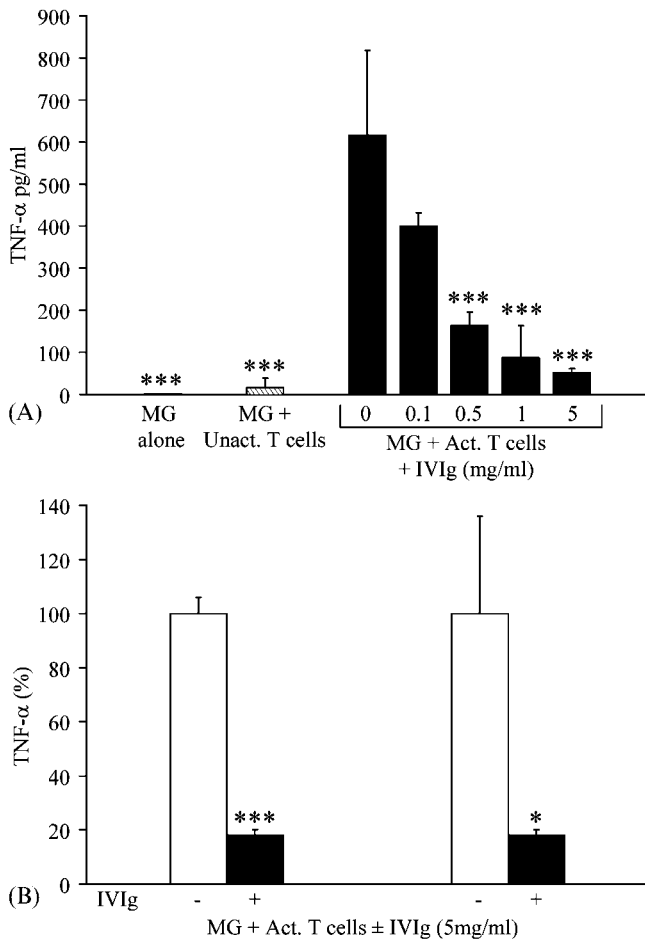


Figure 2: IVIg pre-treatment of activated T cells reduces the TNF- α level that is generated in microglia-T cell activation. In panel (A), concentrations of IVIg above 0.5 mg/ml were effective in alleviating TNF- α levels. *** p <0.001 compared with activated T cells + microglia without any IVIg treatment (one-way ANOVA with Tukey-Kramer multiple comparisons). In panel (B), two other preparations show that IVIg treatment (+) of activated T cells reduced TNF- α resulting from T cell-microglia interaction when compared with controls without (-) IVIg treatment. * p <0.05 and *** p <0.001 compared with corresponding control group (Student's t -test). All values are given as mean \pm SD of triplicates

significant increase in TNF- α levels detected in the culture medium (Figure 1). This was not contributed by the activated T cells alone because we have previously determined that activated T cells by themselves, in plating density of 100,000 cells/well/100 μ l medium, contained barely detectable TNF- α levels^{2,3}. Therefore, there is significant generation of TNF- α when activated T cells come into contact with microglia.

We then addressed if IVIg affects the T cell-microglia interaction. The pre-treatment of T cells with IVIg before their co-culture with microglia was chosen in order to simulate the exposure of T cells to IVIg in the periphery of MS patients before their infiltration into the CNS.

When activated T cell preparations were treated with different concentrations of IVIg 3 hours after their activation with anti-CD3 and before co-culture with microglia, the levels of TNF- α produced in subsequent T cell-microglia interaction were decreased (Figure 2).

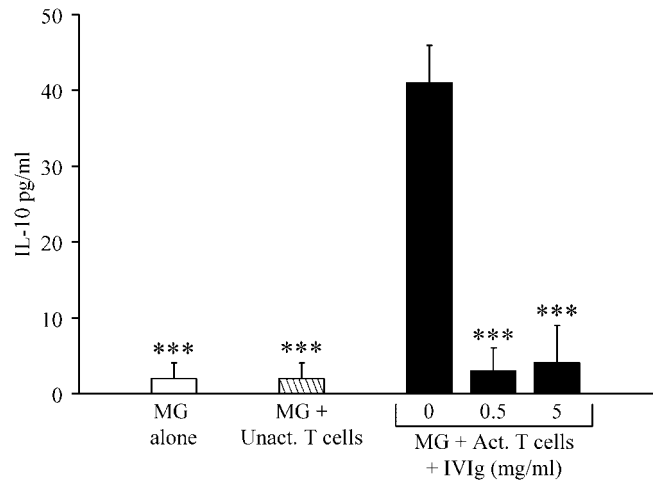


Figure 3: IVIg pre-treatment of activated T cells reduces the IL-10 resulting from microglia-T cell activation. *** p <0.001 compared with activated T cells + microglia without any IVIg treatment (one-way ANOVA with Tukey-Kramer multiple comparisons). All values are given as mean \pm SD of triplicates

There was a dose dependency, in which significance was achieved at IVIg concentrations between 0.5 and 5 mg/ml. The significant reduction of TNF- α when activated T cells were treated with 5 mg/ml IVIg before T cell-microglia interaction was reproduced in two additional sets of experiments (Figure 2B).

We addressed whether another cytokine was also affected by IVIg in T cell-microglia interaction. We selected IL-10 as this cytokine also increases in content when activated T cells come into contact with microglia³. We found that the increased expression of IL-10 that follows the interaction of activated T cells and microglia was attenuated by the IVIg treatment of activated T cells (Figure 3).

In summary, the interaction between activated T cells and microglia increases the production of TNF- α and IL-10. IVIg treatment of activated T cells reduces their subsequent ability to elicit cytokine production in T cell-microglia interaction (Figure 4).

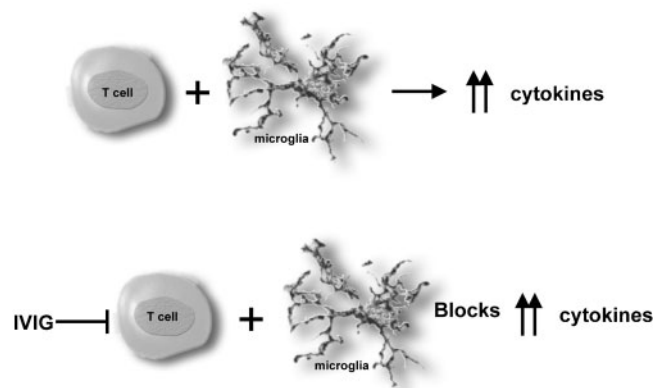


Figure 4: Model of IVIg activation. The top panel shows increased production of cytokines following the interaction of microglia and activated T cells. The exposure of activated T cells to IVIg reduces their subsequent capacity to interact with microglia to elicit cytokine generation (bottom panel)

DISCUSSION

The biology of T cell-microglia interaction is of particular interest in a number of pathologic inflammatory conditions of the brain such as Rasmussen's encephalitis, graft versus host disease, paraneoplastic encephalitis and MS, as these involve infiltration of T cells into the CNS. Once within the CNS, T cells come in close proximity to microglia, innate immune cells that comprise 10–15% of CNS cells. This has been shown in animal models^{5–7} and also in MS, where numerous leukocytes and microglia/macrophages are found in proximity in active demyelinating lesions¹².

It is known that there is reciprocal regulation between microglia and T cells. For example, exposure of microglia to supernatants from Th2 biased T cells alters their impact on the differentiation and cytokine secretion of naive T cells¹³. Similarly, microglia can present antigens to T cells^{14,15}, thereby causing them to be activated or reactivated within the CNS parenchyma. In contrast, one study found that microglia stimulated apoptosis rather than proliferation of a T cell line¹⁶, while another¹⁷ demonstrated that microglia can provoke either antigen-specific T cell anergy or activation, dependent on their state of activation. Antigen-specific T cells can also stimulate microglia, as others have observed^{18,19}.

In this report and reproducing our previous work^{2,3}, we show that T cell-microglia interaction need not require an antigen-specific interaction. So long as T cells have become activated in either syngeneic or allogeneic co-cultures^{2–4}, they can engage microglia cells to lead to the generation of various inflammatory cytokines. In previous work, we determined that TNF- α was generated in T cell-microglia interaction through the engagement of very late antigen (VLA)-4 integrin with vascular cell adhesion molecule-1 (VCAM-1) through CD40-CD40L interaction and the B7 pathway; IL-10 required the interactions of VLA-4 and VCAM-1, and the B7 and CD23 signals^{2,3}.

Treatments that affect T cells in the periphery of patients offer a possibility to alter the interaction of microglia with T cells when the latter infiltrate into the CNS. IVIg is thought to exert its effect mainly on the periphery, even though immunoglobulins may be able to cross the blood-brain barrier to some extent²⁰. IVIg is used as a treatment in autoimmune diseases as Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, graft versus host disease and MS. The mechanisms of action of IVIg in these diseases are still unknown, although they may involve the neutralization of circulating autoantibodies, interference with antigen presentation, the blocking of Fc receptors and the enhancement of remyelination^{21,22}.

In the current manuscript, we have uncovered a new mechanism for IVIg. We show that pre-treatment of activated human adult T cells with IVIg alters T cell reactivity so that these had a decreased capacity to generate measurable TNF- α and IL-10 levels in co-culture of activated T cells and microglia. The concentrations of IVIg used here are relevant, because 1–5 mg/ml amounts are akin to those given therapeutically

in patients (0.4 g/kg, for a 70 kg subject, represents 28 g in a blood volume of 5–6 l).

TNF- α has been implicated as both a beneficial and detrimental factor in immune regulation. TNF- α can induce both apoptotic and non-apoptotic cellular death to neurons and oligodendrocytes^{23,24}, a detrimental outcome, but it can also produce death of activated T cells, the latter being an effect that may limit inflammation. TNF- α can also promote remyelination in the CNS²⁵, in contrast to its lethal effect on oligodendrocytes. IL-10 also has multiple and varied effects on the CNS. It has a role in inducing T cell anergy²⁶ to inhibit the secretion of pro-inflammatory cytokines by different cell types²⁷ and to cause the down-regulation of molecules involved in antigen presentation in macrophages²⁸. In contrast, IL-10 over-expression, in tissues such as the pancreas, can cause inflammation²⁹. Therefore, the impact of IVIg here in reducing levels of both TNF- α and IL-10 may be considered to be one of normalizing effects on T cell-microglia interaction, to result in a normal CNS where most pro- and anti-inflammatory cytokines are not expressed to any extent.

Finally, we wish to emphasize the potential importance of the interaction between activated T cells and microglia as a target for MS therapeutics. We previously reported that the increase of several cytokines in T cell-microglia interaction is reduced not only by interferon- β ^{2,3}, but also by glatiramer acetate³⁰. An experimental treatment in MS, minocycline, also reduces cytokine generation in this interaction⁴. We now add IVIg to the list of agents that dampens cytokine levels. Therefore, our results not only highlight a new mechanism for IVIg, but also highlight the importance of the T cell-microglia interaction as a source of continuous neuroinflammation and a target for drug treatment in MS.

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