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Anterior Chamber-Associated Immune Deviation (ACAID): An Acute Response to Ocular Insult Protects from Future Immune-Mediated Damage?

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Abstract: The "immune privilege" that inhibits immune defense mechanisms that could lead to damage to sensitive ocular tissue is based on the expression of immunosuppressive factors on ocular tissue and in ocular fluids. In addition to this environmental protection, the injection of antigen into the anterior chamber or infection in the anterior chamber induces a systemic suppression of potentially damaging cell-mediated and humoral responses to the antigen. Here we discuss evidence that suggests that Anterior Chamber-Associated Immune Deviation (ACAID)[a] is initiated by an ocular response to moderate inflammation that leads to a systemic immunoregulatory response. Injection into the anterior chamber induces a rise in TNF-α and MCP-1 in aqueous humor and an infiltration of circulating $F4/80^+$ monocytes that home to the iris. The induction of ACAID is dependent on this infiltration of circulating monocytes that eventually emigrate to the thymus and spleen where they induce regulatory T cells that inhibit the inductive or effector phases of a cell-mediated immune response. ACAID therefore protects the eye from the collateral damage of an immune response to infection by suppressing a *future* potentially damaging response to infection.

Keywords: ACAID, immune privilege, inflammation

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Introduction

The "immune privilege" of the *eye in situ* reduces and/or prevents immune defense mechanisms that could lead to damage to the sensitive ocular tissue. This long-recognized protection from "collateral damage" resulting from immune/inflammation reactions is based on the tissue expression of immunosuppressive factors such as Qa-1, fas L and indolamine dioxidase (IDO) expressed by ocular tissues and TGF-β, α–melano-stimulating hormone (α-MSH) and anti-complementary factors in aqueous humor. In addition to this immunosuppressive environment, the injection of antigen into the anterior chamber induces a systemic suppression of cell-mediated responses and complement-fixing IgG antibody to the antigen.[1–4] Moreover, Anterior Chamber-Associated Immune Deviation (ACAID) may also be induced by ocular infection. Individuals with virus-induced acute retinal necrosis do not generate cell-mediated immunity but produce circulating antibody to the virus.[5]

The imposition of ACAID usually occurs one week after the intracameral injection of antigen although twenty-four hr after antigen is injected into the anterior chamber, F4/80$^+$ cells that transfer ACAID when injected intravenously (iv) into naïve mice are recovered from the iris and the peripheral circulation.[6–9]

Six hr after the intracameral injection of ^{125}I- or fluorescein isothiocyanate (FITC)-labeled trinitrophenylated bovine serum albumin antigen, cell-associated antigen is detected in the thymus and spleen although antigen injected iv is detected in the spleen but not the thymus.[8] Within 3 days, thymic NKT cells activated by anterior chamber-induced peripheral blood mononuclear cells (PBMC) emigrate to the spleen where, over the one week period, they participate in the activation of antigen-specific CD4$^+$ and CD8$^+$ regulatory T cells.[7–10] The immunosuppressive ocular environment and mechanisms of self-tolerance to the sensitive ocular tissue such as the retina protects the eye from damaging inflammatory/immune reactions.[1,2] However,since ACAID develops at least one week after induction it is not derived solely from an innate inflammatory response. Moreover, ACAID protects the eye from the collateral damage of a cell-mediated immune reaction by suppressing a *future*, potentially damaging systemic response to infection. Herein we discuss published evidence and evidence our laboratory has obtained that has led us to speculate that ACAID is the result of an ocular response to a limited inflammatory insult.

The Ocular Response to an Intracameral Injection

It has been suggested that the F4/80$^+$ monocytic cells central to the induction of ACAID were resident iris and ciliary body cells that emigrated from the iris via Schlemm's Canal[1,6,10] into the circulation. Because the antigen is present in the eye for at least 24 hr, the antigen could have a "depot" effect although some antigen is in the circulation rapidly after the intracameral injection of the antigen.[8] Because the intracameral injection of antigen induces the appearance of ACAID-inducing PBMC, it is likely that these monocytic cells, rather than circulating antigen alone induce ACAID.

An intravitreal injection of antigen induces an infiltration of monocytic cells to the retina as an early stage of uveitis.[11,12] Moreover, ocular infection induces a strong inflammatory infiltrate in the posterior or anterior chamber.[12–14] Consistent with such an inflammation, there is a rise in inflammatory cytokines in ocular fluids such as aqueous humor. Accordingly, an intracameral injection of particulate antigen induces a rise in mRNA for TNF-α suggesting an early inflammatory response in the anterior chamber.[14,15] Additionally, the systemic suppression of delayed type hypersensitivity is not induced in mice receiving an intracameral injection of antibodies to TNF-α along with antigen suggesting that TNF-α is required during the induction of ACAID. Moreover, the induction of ACAID in these investigations was dependent on the gauge of the needle used for the intracameral injection of soluble proteins and whether the antigen was in a particulate or soluble form.[15] These authors suggested that the intracameral injection of particulate antigens and/or the size of the needle used for intracameral injections induces a trauma required to induce ACAID. Macrophages and dendriform cells expressing the dendritic cell marker CD11c are resident in the iris, ciliary body and choroid in the

anterior segment.[8,10,16,17] Antigen injected into the anterior chamber is taken up rapidly by these cells. Additionally, macrophages and dendriform cells are found near Schlemm's Canal[17,18] believed to be the location of egress of F4/80$^+$ cells from the anterior chamber to the circulation.[1] However, the egress of resident iris cells that engulf antigen to the circulation has been questioned.[16] Soluble antigen rapidly exits the anterior chamber via the circulation and distributes similarly to intravenous antigen.[8] Some of the antigen that exits the eye could be in a tolerogenic form.[19] However, it is likely that the intracameral antigen is presented in the thymus and spleen by so-called "tolerogenic antigen presenting cells" that exited the eye with antigen. Moreover, TGF-β in aqueous humor or produced by iris F4/80$^+$ cells induces a suppressive phenotype in peripheral F4/80$^+$ cells[10,20,21] suggesting that the induction of a suppressive phenotype in F4/80$^+$ cells occurs in anterior chamber.

Similar to the treatment of peripheral F4/80$^+$ cells with aqueous humor and antigen, the incubation of F4/80$^+$ peritoneal exudate cells with antigen and TGF-β *in vitro* imposes a suppressive phenotype on the cells. When these cells are injected iv into naive mice they induce antigen-specific splenic regulatory T cells similar to that obtained by the intracameral injection of antigen.[21] ACAID is induced by very few F4/80$^+$ cells treated in this manner[1] suggesting that (undetectable) cells emigrating from the iris and ciliary body may induce ACAID. Moreover, F4/80$^+$ iris cells recovered from mice that received an intracameral injection of antigen induce the induction of ACAID or confer on F4/80$^+$ PBMC a suppressive phenotype similar to those circulating cells recovered from mice that received an intracameral injection of antigen.[9,21] It is likely that antigen taken up and processed by iris antigen presenting cells could be transferred to the PBMC that infiltrated the anterior chamber much the same way that antigen is transferred to ACAID-inducing B cells in the spleen by TGF-β-treated, antigen-bearing peritoneal exudate cells.[22] Taken together, these observations suggest that events occurring within the anterior chamber could induce a suppressive phenotype on non-resident inflammatory cells that infiltrated the anterior chamber.

Based on the above considerations, we investigated the possibility that the intracameral *injection* of antigen induces an influx of circulating PBMC to the anterior chamber due to the trauma of injection. These infiltrated cells would be exposed to immunosuppressive TGF-β in aqueous humor and resident iris/ciliary body dendriform cells that could present the injected antigen to the infiltrated PBMC. Contingent with this hypothesis, the cells that were recruited to the anterior chamber due to the injury caused by the injection then recirculate and home to the thymus and spleen where they induce regulatory T cells.

Within 3 hr after the intracameral injection of ovalbumin (OVA) we also found a rise of TNF-α in aqueous humor as described by Ferguson et al.[15] Additionally, we have observed a rise in the chemokine MCP-1 in aqueous humor six hr after the intracameral injection (Fig. 1). A needle stick without injection of PBS induced the appearance of TNF-α in aqueous humor and the injection of PBS only increased the amount of TNF-α five fold more than that obtained by a needle stick. TNF-α in aqueous humor decreases rapidly and is not detected 12 hr after the intracameral injection of ovalbumin (OVA) (data not shown). MCP-1 is maintained in aqueous humor for a longer period. However, unlike TNF-α, peak levels of MCP-1 are reached in aqueous humor by 12 hr and remain until 16 hr after the intracameral injection of OVA (data not shown). This rise in TNF-α and MCP-1 levels in aqueous humor after the intracameral injection of antigen suggests the initiation of an inflammatory response in the anterior chamber. Moreover, TNF-α is not increased in aqueous humor nor is ACAID induced if the needle used for the intracameral injection is too small and/or the antigen is not inflammatory.[15]

To determine whether there is a concomitant influx of inflammatory cells into the anterior chamber following the intracameral injection of antigen, mice injected iv with PBMC labeled with the fluorescent dye CFSE also received an intracameral injection. Using this approach we observed a significant influx of CFSE-labeled and unlabeled (host) F4/80$^+$ monocytic cells that express the chemokine receptors CCR2 and CCR5 as shown by a recovery of these cells from the irides of mice 24 hr after the

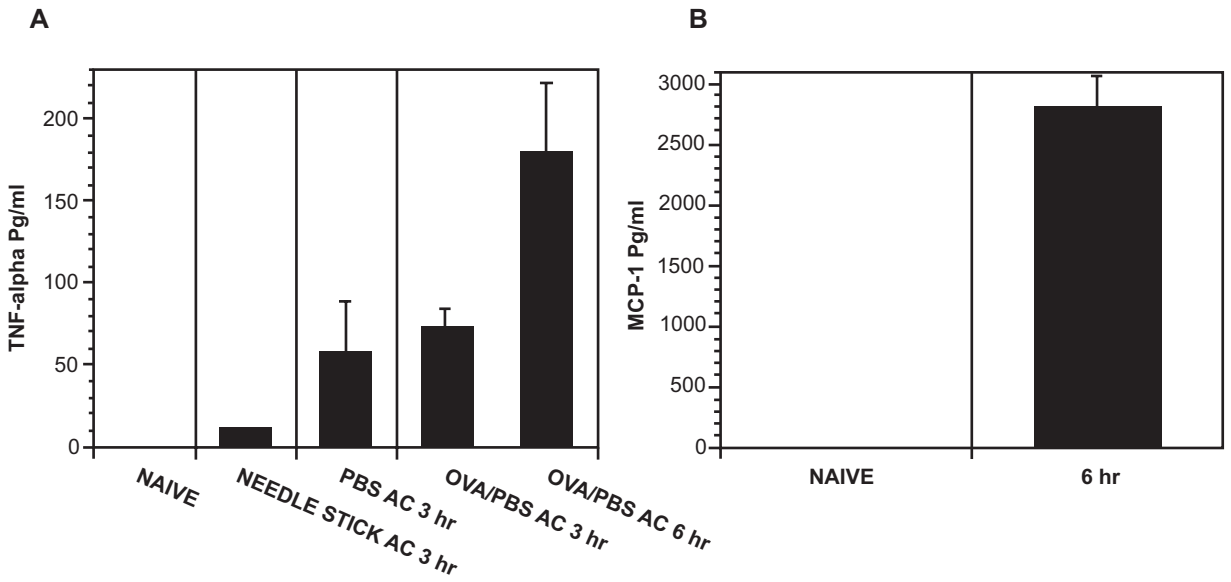

Figure 1. The intracameral injection of antigen elevates TNF-a and MCP-1 in aqueous humor. Three–6 hr after 6–8 week-old naïve female BALB/c mice received iv $5 \times 10^6 - 1 \times 10^7$ CFSE-labeled PBMC the mice were anesthetized with an ip injection of ketamine/xylazine[7] and received an intracameral injection of PBS, PBS + 50 μg OVA or a needle stick only with a 24 g needle. Six hr after naïve mice received an intracameral injection, aqueous humor was recovered from the euthanized mice and 10 μl assayed by ELISA for TNF-α and MCP-1. Data represent the average +/– S.E.M. pg detected from 4–6 replicates/group in 2–3 experiments.

mice received an intracameral injection of antigen. There was no increase in these cells in controls that received iv CFSE-labeled PBMC but did not receive an intracameral injection of antigen.[23] Approximately 48 hr after the intracameral injection of antigen, the number of infiltrated cells at the iris decreases and increases in the thymus and spleen (Fig. 2). This increase in circulating monocytic cells at the iris is likely due to an infiltration of circulating monocytes in response to the trauma of the intracameral injection +/– the irritant of PBS and/or antigen/PBS because the number of infiltrated monocytic cells increased when the mice received intracameral antigen: infiltration of CFSE-labeled PBMC induced by injection only was <injection of PBS < injection of TNP-BSA/PBS. Monocytic cells recruited to the iris after the intracameral injection of antigen express both CCR2 and CCR5.[23] Migration of the cells to the iris requires the expression of CCR2 but not CCR5 because CCR2 –/– PBMC do not home to the iris but CCR5 –/– PBMC home to the iris after the intracameral injection of antigen. Moreover, the intracameral injection of OVA into CCR2 –/– mice does not induce circulating monocytic cells that transfer the suppression of delayed-type hypersensitivity (DTH) to OVA when injected iv into wildtype recipients.[23]

The Induction of ACAID Depends on Cytokines in Aqueous Humor

ACAID is transferred to naïve mice by the iv injection of F4/80$^+$ peritoneal exudate cells or PBMC treated with antigen and aqueous humor or cells recovered from the iris/ciliary bodyof mice that received intracameral antigen[1,7,9,10,21] Moreover, TGF-β in aqueous humor or produced by the iris cells is responsible for the induction of the immunosuppressive properties of the F4/80$^+$ cells. These *in vitro* observations suggest that TGF-β induces physiological changes in these macrophages, thereby "converting" them to a suppressor-inducer phenotype. Therefore, such events likely occur to F4/80$^+$ circulating cells when these cells infiltrate the anterior chamber.

Mild Inflammation Induced by the Injection of Antigen Induces ACAID

ACAID is not induced in mice that received an intracameral injection of antigen and antibodies to TNF-α.[15] As an extension of these investigations we have observed that the intracameral injection of antibodies to MCP-1 or TGF-β with antigen

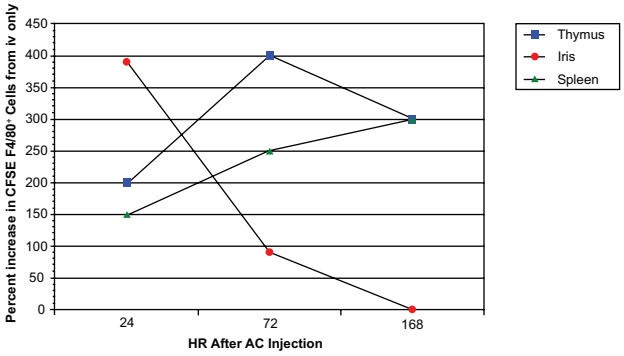

Figure 2. Migration of PBMC after intracameral injection. Anesthetized[7] BALB/c mice received an intracameral injection of trinitrophenylated bovine albumin six hr after the mice received iv $5 \times 10^6 - 1 \times 10^7$ CFSE-labeled PBMC. Twenty-four hrs after the intracameral injection the mice were euthanized by CO_2 inhalation and irides, spleens and thymi removed, pooled and single cell suspensions prepared. The cells were then labeled with phycocyanin-anti-F4/80 and analyzed by flow cytometry. The percent increase in CFSE, F480+ cells was computed by:

$$\frac{\%\ \text{CFSE, F480+ cells in iv, AC group}}{\%\ \text{CFSE, F480+ cells in iv only group}} \times 100$$

also prevents the induction of ACAID and the production of circulating, ACAID-inducing PBMC. However, ACAID can be "rescued" in mice receiving intracameral antigen and anti-TGF-β if they also receive an iv injection of PBMC recovered from other mice that received an intracameral injection of antigen only.[24] These observations suggest that the antibodies injected into the anterior chamber are inhibiting the induction of ACAID-inducing monocytes in the anterior chamber itself before these cells exit the anterior chamber and emigrate to the thymus and spleen. We have observed that CCR2 is required for PBMC to enter the anterior chamber after the intracameral injection of antigen. However, MCP-3 and MCP-5 are also ligands for CCR2. Therefore, these chemokines may also attract F4/80+ PBMC to the anterior chamber. Additionally, the binding of MCP-1 to it's receptor might also initiate events necessary to "convert" these cells to a suppressive phenotype by affecting the activity of the cell after binding to the CCR2.[25] F4/80+ peritoneal exudate cells treated with TGF-β and antigen have the same ACAID-inducing properties as PBMC recovered from mice that received an intracameral injection of antigen.[10,20,21,26,27] In fact, aqueous humor or culture supernatants recovered from F4/80+ cells from the iris or ciliary body has the same TGF-β-dependent effect. This influence of TGF-β may depends on signal transduction mediated by PI 3 kinase that phosphorylates Akt participating in signal transduction[28,29] because the intracameral injection of the pan PI 3 kinase inhibitor wortmannin with antigen prevents the induction of ACAID.[24] The PI 3 kinase/Akt pathway in monocytes controls an excessive inflammatory phenotype involving the production of IL-10.[29] Therefore, there may be a feedback loop involving this pathway where TGF-β and IL-10 reinforce each other maintaining a highly active phosphorylated Akt. Consistent with these observations is the requirement for both TGF-β and IL-10 for the induction of F4/80+ peritoneal exudate cells to induce CD8+ regulatory T cells.[30] Although these cells may produce both TGF-β and IL-10, regulatory thymic NKT cells and CD8+ spleen T cells resistant to TGF-β could be induced after the intracameral injection of antigen.[31] Moreover, thrombospondin, an active participant in inflammation[32] is required for the activation of tolerogenic macrophages by TGF-β.[33]

In aggregate, events induced by an insult in the anterior chamber (*vide supra*) suggest that the experimental *injection* of antigen into the anterior chamber or a naturally occurring infection induces a response to the trauma (Table 1, Fig. 3) characterized by:

- The production of inflammatory cytokines and chemokines (TNF-α, MCP-1) (due to the release and/or activation of Damage-Associated Molecular Patterns, DAMPS). TGF-β (in aqueous humor) promotes the production of TNF-α TNF-α may augment the production of MCP-1 and the expression of ICAM-1 on the vascular endothelium.
- Circulating F4/80+ cells recruited to the anterior chamber.
- Interactions between the infiltrated monocytes and iris/cililary body antigen presenting cells resulting in the transfer of antigen to the infiltrated PBMC.
- TGF-β-mediated "conversion" of the infiltrated, antigen-bearing PBMC to a suppressive phenotype.
- The egress of the infiltrated, now suppressor-inducer PBMC via the Canal of Schlemm to the thymus and spleen where they participate in the induction of regulatory T cells.

Table 1. Manipulation of the immune response and ACAID by intracameral injection.

Injection into the anterior chamber	DTH suppression	AH* MCP-1	AH TNF-α	AC-F4/80$^+$ cells in blood
Antigen (Ag)	+ve	+ve	+ve	+ve
Ag + TGF-β	–ve; +ve with AC-PBMC rescue	+ve	+ve but reduced	–ve
Ag + TNF-α	–ve; +ve with AC-PBMC rescue?	+ve; May be reduced	–	N.T
Ag + MCP-1	–ve	–	N.T	N.T
Ag + wortmannin (PI 3 Kinase inhibitor)	–ve; May be +ve with ACPBMC rescue?	+ve	+ve	N.T
Ag + CCR2 -/-mice	no circulating PBMC	N.T	N.T	–ve

Abbreviations: AC, anterior Chamber; AH*, Aqueous Humor after the injection injury; TGF-β, blocking antibody; MCP-1, blocking antibody; TNF-α, blocking antibody; AC-PBMC rescue, Intravenous injection of 24 hours PBMC fraction from AC antigen injected animals restores DTH suppression; N.T, not tested.

Taken together, the induction of immunoregulatory T cells via a (moderate) inflammation depends on cellular and environmental factors. Too much inflammation will abrogate this effect[34] indicating that intense infection or autoimmune reactions will block both environmental immune privilege and the induction of systemic immunosuppression. Thus, ACAID appears to be a "pre-emptive strike" by the immune system that will protect against a future, damaging immune response. This raises the question of whether moderate

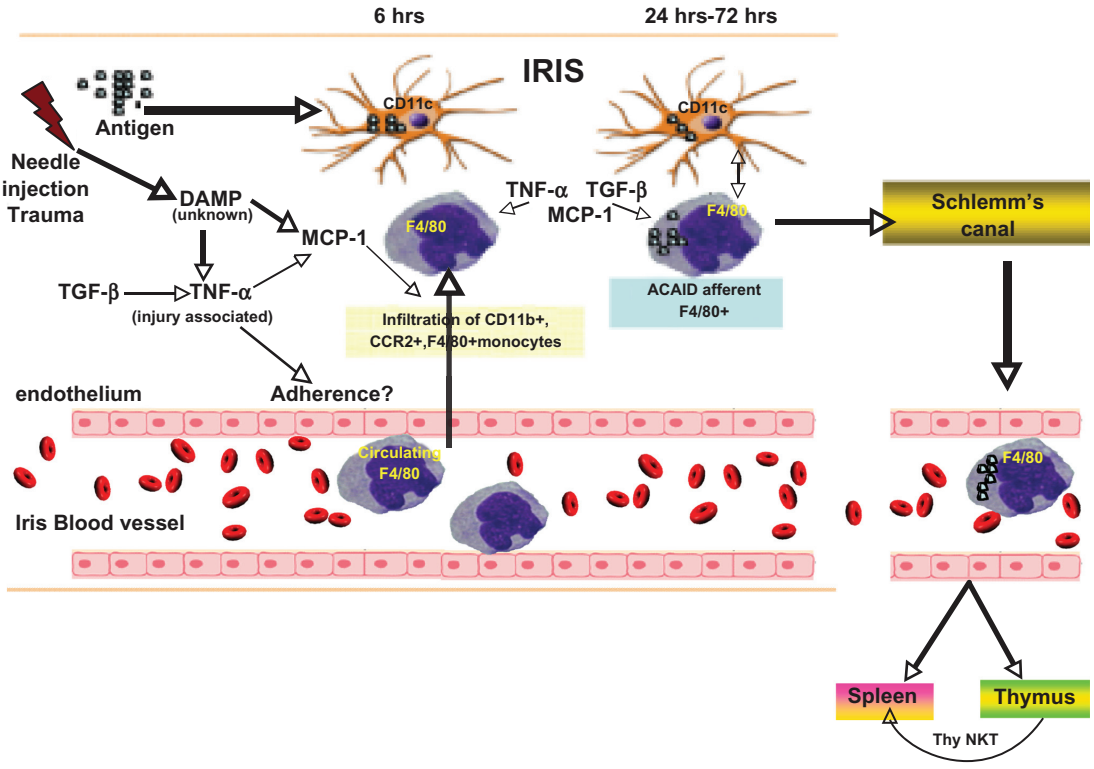

Figure 3. Hypothetical model for events in the anterior chamber following the intracameral injection of antigen. The trauma of injection induces damage associated molecular pattern (DAMP) molecules that induce the production of MCP-1 and TNF-α. TNF-a is also induced and/or maintained by TGF-β in aqueous humor. TNF-a increases the production of MCP-1. MCP-1 attracts circulating F4/80$^+$ cells that enter the anterior chamber and obtain antigen from resident iris/ciliary body F4/80$^+$,CD11c$^+$ cells. The infiltrated monocytes are influenced by TGF-β and exit the anterior chamber via Schlemms canal. These cells recirculate to the thymus and spleen where they participate in the induction of regulatory thymocytes and splenic T cells.

inflammation at other immune-privileged sites (e.g. the brain, testes, gut) might also induce regulatory T cells via the infiltration and conversion of monocytic cells that in turn induce regulatory T cells.

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Abbreviations

ACAID,[a] anterior chamber-associated immune deviation; CFSE, carboxyfluorescein succinimidyl methyl ester; DAMP, damage-associated molecular pattern; DTH, delayed-type hypersensitivity; IDO, indolamine dioxidase; iv, intravenously; α–MSH, alpha melanocyte stimulating hormone; NKT, natural killer T cell; OVA, ovalbumin; PBMC, peripheral blood mononuclear cell; TGF-β, transforming growth factor β; TNF-α, Tumor necrosis factor-α.

Disclosures

The authors report no conflicts of interest.

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