

Intravenous immunoglobulin preparations contain anti-Siglec-8 autoantibodies

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Background: Human intravenous immunoglobulin (IVIg) preparations are used for the treatment of autoimmune and allergic diseases. Natural autoantibodies are believed to contribute to IVIg-mediated anti-inflammatory effects.

Objective: To address the question of whether IVIg preparations contain anti-sialic acid-binding Ig-like lectin-8 (anti-Siglec-8) autoantibodies.

Methods: The presence of possible anti-Siglec-8 autoantibodies in IVIg preparations was first examined by functional eosinophil death and apoptosis assays. Specificity of IVIg effects was shown by depleting anti-Siglec-8 autoantibodies from IVIg. Binding of purified anti-Siglec-8 autoantibodies to recombinant Siglec-8 was demonstrated by an immunodot assay.

Results: IVIg exerts cytotoxic effects on purified human blood eosinophils. Both potency and efficacy of the IVIg-mediated eosinophil killing effect was enhanced by IL-5, granulocyte/macrophage colony-stimulating factor, IFN- γ , TNF- α , and leptin. Similarly, inflammatory eosinophils obtained from patients suffering from the hypereosinophilic syndrome (HES) demonstrated increased Siglec-8 cytotoxic responses when compared with normal blood eosinophils. Pharmacologic blocking experiments indicated that the IVIg-mediated additional eosinophil death in the presence of cytokines is largely caspase-independent, but it depends on reactive oxygen species. Anti-Siglec-8 autoantibody-depleted IVIg failed to induce caspase-independent eosinophil death.

Conclusion: IVIg preparations contain natural anti-Siglec-8 autoantibodies.

Clinical implications: Anti-Siglec-8 autoantibodies present in IVIg preparations may have therapeutic relevance in autoimmune and allergic diseases, respectively, such as Churg-Strauss syndrome. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Apoptosis, autoantibodies, eosinophils, inflammation, IVIg, Siglec-8

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Abbreviations used

DPI:	Diphenyleneiodonium
HES:	Hypereosinophilic syndrome
IVIg:	Intravenous immunoglobulin
MIF:	Macrophage migration inhibitory factor
NAC:	N-Acetyl-L-cysteine
NADPH:	Nicotinamide adenine dinucleotide phosphate
ROS:	Reactive oxygen species
Siglec:	Sialic acid-binding immunoglobulin-like lectin
VAD:	N-benzyloxycarbonyl
	(z)-Val-Ala-Asp-fluormethylketone (fmk)

Originally used as a replacement treatment in humoral immunodeficiencies, intravenous immunoglobulin (IVIg) preparations are to date also used in a variety of chronic inflammatory disorders.¹⁻³ Despite a broad range of potential activities, the exact mechanisms of the immunoregulatory effects of IVIg are still unclear, even for approved indications. Therefore, a better understanding of the mechanisms of action of IVIg is essential for a more rational use of these drugs.

One potential mechanism responsible for the anti-inflammatory effects of IVIg preparations is the cytotoxic effect on inflammatory cells. For instance, we and others reported that IVIg contain naturally occurring autoantibodies against the death receptor Fas.^{4,5} Moreover, anti-sialic acid-binding immunoglobulin-like lectin-9 (anti-Siglec-9) autoantibodies, which trigger both caspase-dependent and caspase-independent cell death pathways in neutrophils, were recently identified in IVIg.⁶

Siglecs belong to the immunoglobulin supergene family and have been shown to activate death pathways, at least in granulocytes. For instance, Siglec-8 ligation on blood eosinophils induced apoptosis.^{7,8} Similarly, ligation of Siglec-9 triggered apoptosis in neutrophils.⁹ Interestingly, these cytotoxic effects were enhanced in the presence of cytokines and, at least partially, caspase-independent.^{7,9} This phenomenon was also observed when neutrophils were treated with IVIg *in vitro*, which led to the identification of natural anti-Siglec-9 autoantibodies in IVIg.⁶

Here we show a cytotoxic effect of IVIg on eosinophils that is remarkably enhanced in the presence of IL-5,

GM-CSF, IFN- γ , TNF- α , and leptin. An increased cytotoxic effect of IVIg was also observed in eosinophils obtained from patients with the hypereosinophilic syndrome (HES) when compared with normal eosinophils. Subsequent analysis revealed that agonistic autoantibodies directed against Siglec-8 are responsible for the cytokine-dependent cytotoxic effect of IVIg.

METHODS

Cells

Peripheral blood eosinophils were purified from control individuals and HES patients as described previously.¹⁰⁻¹² The resulting cell populations contained less than 2% contaminating cells. Cell purity was assessed by staining with Diff-Quik (Medion GmbH, Düringen, Switzerland) and light microscopy analysis. Written informed consent was obtained from all blood donors, and the study was approved by the ethics committee of the Canton Bern.

IVIg preparations

The IVIg preparation used in this study was Sandoglobulin (ZLB Behring AG, Bern, Switzerland). To remove the stabilizing sucrose component, the IVIg preparation was dialyzed with a Spectra/Por 3 membrane (standard cellulose dialyze tubing; molecular weight cutoff, 3500 Da) obtained from Spectrum Medical Industries (Los Angeles, Calif). F(ab')₂ fragments of IVIg preparations were produced by digestion and subsequent purification using a protein A-agarose column and an ImmunoPure F(ab')₂ preparation kit (Pierce Chemical, Rockford, Ill). The purity of the F(ab')₂ fragments was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis. Depletion of anti–Siglec-8 and anti–Siglec-3 antibodies from IVIg preparations was performed by affinity chromatography.⁶ For this purpose, a recombinant Siglec-8/Fc fusion protein was produced as described previously,¹³ and Siglec-3/Fc was obtained commercially (R&D Systems, Abingdon, United Kingdom). The content of aggregate formation was tested by high-performance liquid chromatography and was found to be less than 2% for all IVIg fractions.

Cell cultures

Eosinophils were cultured at 1×10^6 /mL in the presence or absence of cytokines, antibodies, or both, for the indicated times using complete culture medium (RPMI 1640 containing 10% fetal calf serum and 200 IU/mL penicillin/100 μ g/mL streptomycin, all from Life Technologies, Basel, Switzerland). If not indicated otherwise, cells were stimulated with 20 mg/mL of IVIg ($= 133.3 \mu\text{mol/L}$). F(ab')₂ fragments of IVIg were used at $133.3 \mu\text{mol/L}$. Fc γ receptors were blocked with a mixture of antihuman monoclonal antibody (mAb) F(ab')₂ fragments from anti-CD32 (clone 7.3) and anti-CD64 (clone 10.1) (both from Ancell Corp., Bayport, Minn). These mAb F(ab')₂ fragments were used at 600 nmol/L and added 30 minutes before the addition of IVIg.⁶ Cytokine stimulation occurred 25 minutes before the addition of IVIg. GM-CSF (Novartis Pharma GmbH, Nürnberg, Germany), IL-5 (R&D Systems), TNF- α (R&D Systems), and IL-1 β (R&D Systems) were used at 25 ng/mL. IFN- γ (R&D Systems) was used at 85 ng/mL, and leptin (R&D Systems) at 0.5 $\mu\text{mol/L}$. Macrophage migration inhibitory factor (MIF) was produced as a maltose binding protein fusion protein as previously described¹⁴ and used at 7.5 $\mu\text{g/mL}$. The caspase inhibitor N-benzyloxycarbonyl (z)-Val-Ala-Asp (VAD)-fluormethylketone (fmk) (BD Biosciences, Basel, Switzerland) was used at 50 $\mu\text{mol/L}$. N-Acetyl-L-cysteine (NAC) (Sigma-Aldrich, Buchs, Switzerland) was used at 10 $\mu\text{mol/L}$. For nicotinamide adenine dinucleotide

phosphate (NADPH) oxidase-inhibition experiments, eosinophils were incubated with 20 $\mu\text{mol/L}$ diphenyleneiodonium (DPI) (Calbiochem-Novabiochem Corp., La Jolla, Calif) at 37°C for 5 minutes and then washed twice with RPMI 1640 before culture.

Determination of cell death and apoptosis

Eosinophil death was assessed by uptake of 1 $\mu\text{mol/L}$ ethidium bromide and flow cytometric analysis (FACS Calibur; BD Biosciences).^{15,16} To determine whether cell death was apoptosis, DNA fragmentation was measured.^{16,17}

Immunodot assay

The binding of total and anti–Siglec-8–depleted IVIg to Siglec-8 was determined by a dot assay on nitrocellulose (Millipore, Bedford, United Kingdom). Recombinant glutathione-S-transferase (GST)-tagged Siglec-8 (Abnova Corp., Taipei City, Taiwan) in phosphate-buffered solution (PBS) was dotted at 50 $\mu\text{g/mL}$ on nitrocellulose filters (1 $\mu\text{L/dot}$). Each dot was blocked with PBS containing 0.15% casein (PBS-C) and then incubated overnight at 4°C with 10 $\mu\text{g/mL}$ complete IVIg or anti–Siglec-8–depleted IVIg in PBS-C. As controls, dots were also incubated with anti–Siglec-8 mAb (Abnova Corp.; 1:500) and anti–Siglec-3–depleted IVIg. After washing with PBS containing 0.1% Tween 20, bound antibodies were detected with horseradish peroxidase–conjugated goat antihuman IgG (Fc) (Nordic, Tilburg, The Netherlands) or goat antimouse IgG (Fc) (Jackson ImmunoResearch Laboratories, West Grove, Pa; 1:1000) in PBS-C. The dots were soaked in enhanced chemiluminescence detection reagents and visualized using the luminescent image analyzer Las-3000 (Fujifilm, Tokyo, Japan).

Statistical analysis

Statistical analysis was performed by using the Student *t* test. If mean levels are presented, the standard error of the mean (SEM) and the number (*n*) of independent experiments are indicated in each case. A probability value of less than .05 was considered statistically significant.

RESULTS

IVIg induces eosinophil death, which is accelerated by IL-5, GM-CSF, and leptin

As we have previously observed cytotoxic effects of IVIg on neutrophils,^{5,6} we assessed in this study IVIg-mediated eosinophil death in the presence and absence of IL-5 *in vitro*. Incubation of eosinophils with IVIg resulted in accelerated loss of viability (Fig 1, A) and concentration-dependent cell death (Fig 1, B). Strikingly, we found that simultaneous exposure of the cells to the eosinophil survival factor IL-5 remarkably increased IVIg-mediated cytotoxicity (Fig 1, B). Similarly to previously published work in neutrophils,^{6,9} this increased cytotoxicity was most efficient if cells were preincubated with IL-5 for 25 minutes before IVIg was added. We also tested additional cytokines for their capacity to enhance IVIg-mediated cytotoxicity in eosinophils. Similarly to IL-5, preincubation with the eosinophil survival factor GM-CSF increased eosinophil death. Leptin, another eosinophil survival factor,¹⁸ as well as IFN- γ and TNF- α , also enhanced IVIg-mediated cytotoxicity, although with less efficacy when compared with IL-5 or GM-CSF (Fig 1, C). In contrast, IL-1 β and MIF did not prime the IVIg-initiated death pathway.

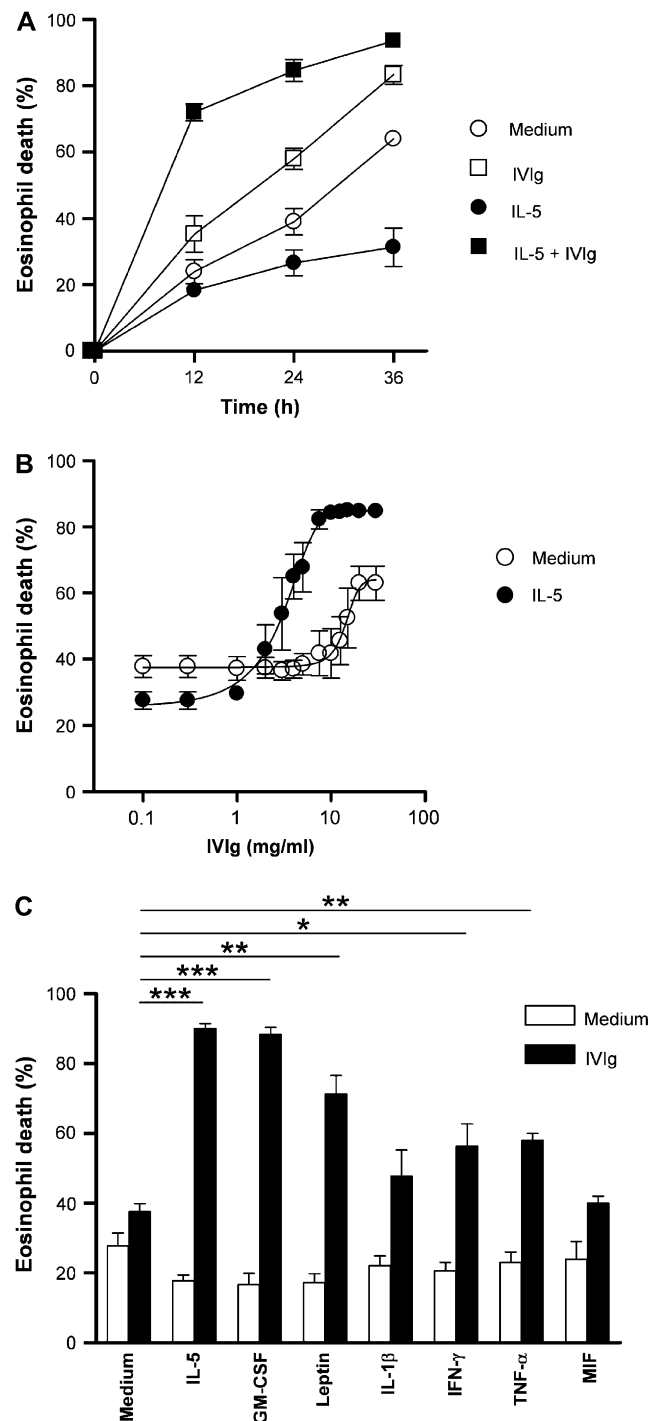


FIG 1. IVIg accelerates spontaneous eosinophil death, which is enhanced after priming with IL-5, GM-CSF, IFN- γ , TNF- α , or leptin. **A**, Time-dependent acceleration of eosinophil death by treatment with IVIg in the presence and absence of IL-5. **B**, Concentration-effect curve of IVIg in 24-hour eosinophil cultures. IL-5, at low concentrations of IVIg, partially blocked cells from spontaneous death, whereas it promoted death at higher concentrations. **C**, IL-5, GM-CSF, IFN- γ , TNF- α , and leptin, but not IL-1 β and MIF, enhanced IVIg-mediated eosinophil death in 24-hour cultures. In all panels, means $\pm$ SEMs of 4 independent experiments are shown. * $P < .05$; ** $P < .01$; *** $P < .001$.

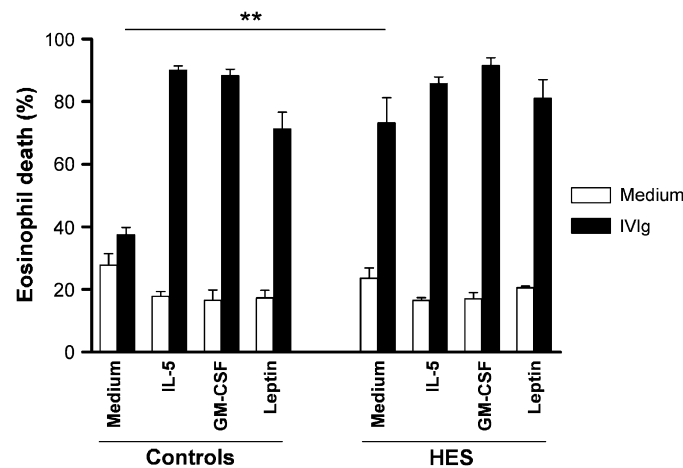


FIG 2. Eosinophils from patients with HES demonstrate increased IVIg-mediated cell death susceptibility. Although spontaneous death of HES eosinophils was delayed, IVIg-mediated death was accelerated. Additional *in vitro* priming with IL-5, GM-CSF, or leptin had no effect on these inflammatory eosinophils, which suggests that blood eosinophils from patients with HES were primed *in vivo*. Results (means $\pm$ SEMs) of 24-hour cultures are shown (control individuals: $n = 4$; HES patients: $n = 5$). ** $P < .01$.

Enhanced IVIg-mediated eosinophil death associated with hypereosinophilic syndrome

As eosinophils are exposed to IL-5 in most cases of HES,^{12,19} we hypothesized that eosinophils derived from HES patients may respond with an enhanced death rate after IVIg treatment *in vitro*. Indeed, compared with normal blood eosinophils, HES eosinophils exhibited increased susceptibility toward IVIg-mediated death that was not further increased by preincubation with IL-5, GM-CSF, or leptin (Fig 2).

IVIg activates both caspase-dependent and caspase-independent death pathways in eosinophils

We next investigated whether the IVIg-mediated eosinophil death is caspase-dependent. Caspases play a critical role in the execution phase of eosinophil apoptosis.²⁰ In the absence of cytokines, the pan-caspase inhibitor z-VAD-fmk completely blocked IVIg-mediated cell death, which suggests that IVIg triggers apoptosis under these conditions (Fig 3, A). Indeed, IVIg induced redistribution of phosphatidyl serine (data not shown), which is a characteristic feature of apoptotic eosinophils.¹⁸ In contrast, the enhanced IVIg-mediated eosinophil death in the presence of IL-5 was only partially blocked by z-VAD-fmk (Fig 3, A), which suggests the involvement of caspase-independent death mechanisms at least in a subpopulation of dying eosinophils. Interestingly, IVIg-mediated cell death in HES eosinophils was also only partially dependent on caspases (Fig 3, B). This observation points to the possibility that HES eosinophils had been exposed to IL-5 and/or other cytokines *in vivo* that primed these cells for additional caspase-independent death after IVIg stimulation.

Reactive oxygen species (ROS) have previously been described as regulators of both apoptosis and necrosis

in several cellular systems.²¹ NAC, a well-characterized ROS scavenger, reduced the cytotoxic effect of IVIg in the presence of IL-5 and completely blocked it in the absence of cytokines (Fig 3, C). In circulating phagocytes, such as eosinophils, ROS are mostly generated by the inducible NADPH oxidase system. To further investigate the potential role of ROS in IVIg-mediated eosinophil death, we used the NADPH oxidase-inhibitor DPI. Blocking of ROS synthesis completely abrogated IVIg-mediated eosinophil death in both the presence and the absence of IL-5 (Fig 3, C). Collectively, these data suggest a role for ROS in both caspase-dependent and caspase-independent forms of cell death induced by IVIg in eosinophils.

To exclude the possibility that the cytotoxic effects of IVIg were caused by binding to Fc receptors, which are present on the surface of eosinophils, we used F(ab')₂ fragments of IVIg⁶ that demonstrated the same cytotoxic effect as complete IVIg. Moreover, avoiding binding to Fcγ receptors using a mixture of mAb F(ab')₂ fragments^{6,22} did not prevent IVIg-mediated eosinophil death in the presence or absence of IL-5 (Fig 3, D). These data suggested that IVIg contain antibodies, which somehow bind surface structures on eosinophils and subsequently promote eosinophil death.

Autoantibodies against Siglec-8 are responsible for the increased cytotoxicity of IVIg in the presence of IL-5

It has been reported that Siglec-8 ligation on eosinophils induces apoptosis and that such a treatment in the presence of IL-5 is associated with enhanced cell death.⁷ Moreover, Siglec-9 ligation resulted in the activation of both caspase-dependent and caspase-independent death pathways in the presence of GM-CSF in neutrophils.⁹ Given that we previously obtained evidence for the

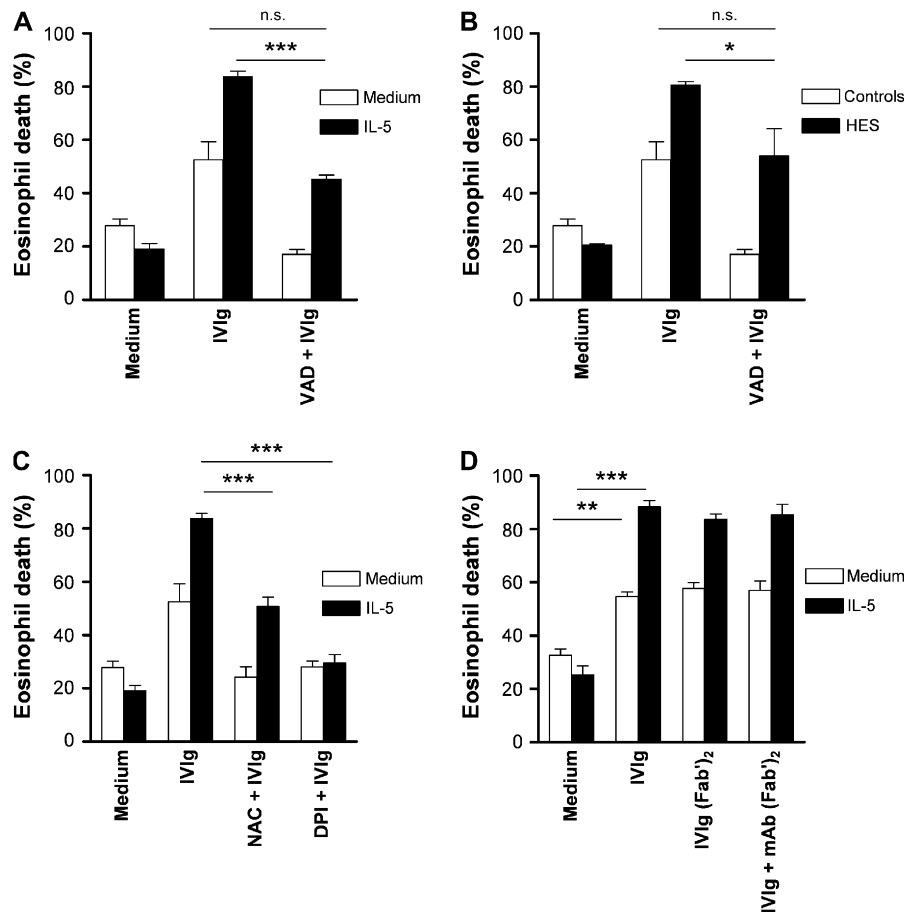


FIG 3. The death mediated by IVIg triggers caspase-dependent and caspase-independent death pathways that both involve ROS but not Fc γ receptors. The caspase inhibitor z-VAD-fmk completely blocked IVIg-mediated eosinophil death. However, the enhanced IVIg-triggered death after IL-5 priming was only partially blocked by this inhibitor (**A**). Similarly, z-VAD-fmk only partially blocked IVIg-triggered death in HES eosinophils (**B**). The ROS scavenger NAC completely blocked IVIg-triggered eosinophil death in the absence of IL-5 but only partially in the presence of IL-5. The inhibitor of ROS production DPI completely blocked IVIg-triggered eosinophil death in both the absence and the presence of IL-5 (**C**). Acceleration of eosinophils death by treatment with IVIg or F(ab')₂ fragments of IVIg. IVIg effects were also determined in the presence of mAb F(ab')₂ fragments to avoid binding to Fc γ receptors (**D**). In all panels, means $\pm$ SEMs of 4 independent experiments are shown. * $P < .05$; ** $P < .01$; *** $P < .001$.

presence of natural anti-Siglec-9 autoantibodies in IVIg able to trigger cell death in neutrophils,⁶ we hypothesized that the IVIg-mediated death in eosinophils is, at least partially, mediated by natural anti-Siglec-8 autoantibodies. To identify these antibodies, we applied an immunodot assay to investigate the binding capacity of IVIg to a recombinant Siglec-8 protein. Incubation of IVIg revealed a positive signal, which suggests that they indeed contain antibodies directed against Siglec-8. As a positive control, we used an anti-Siglec-8 mAb in this system. Anti-Siglec-8-depleted IVIg showed markedly reduced binding to Siglec-8 compared with total IVIg, which demonstrates the specificity of the assay. Anti-Siglec-3-depleted IVIg was used as an additional control and found to bind to Siglec-8 in the same intensity as total IVIg did (**Fig 4, A**).

We next investigated the death-inducing capacity of the IVIg fraction depleted of anti-Siglec-8 autoantibodies. In the absence of IL-5, normal and anti-Siglec-8-depleted

IVIg demonstrated the same death efficacy. In contrast, the strong death-inducing capacity of IVIg in the presence of IL-5 was essentially absent if anti-Siglec-8-depleted IVIg was used (**Fig 4, B**). To further demonstrate that the enhanced eosinophil death in the presence of IL-5 depends on anti-Siglec-8 autoantibodies, we analyzed DNA fragmentation using a flow cytometric assay. DNA fragmentation was similarly enhanced on treatment with IVIg and anti-Siglec-8-depleted IVIg. In the presence of IL-5, the generation of hypoploid DNA by IVIg was further increased. This enhanced DNA fragmentation, however, was largely absent if the anti-Siglec-8-depleted IVIg fraction was used. Similarly, preincubation of IVIg with recombinant Siglec-8 also reduced the additional formation of hypoploid DNA in the presence of IL-5. In contrast, the same pretreatment had almost no effect on DNA fragmentation in the absence of IL-5, which suggests that Siglec-8-independent death mechanisms, such as

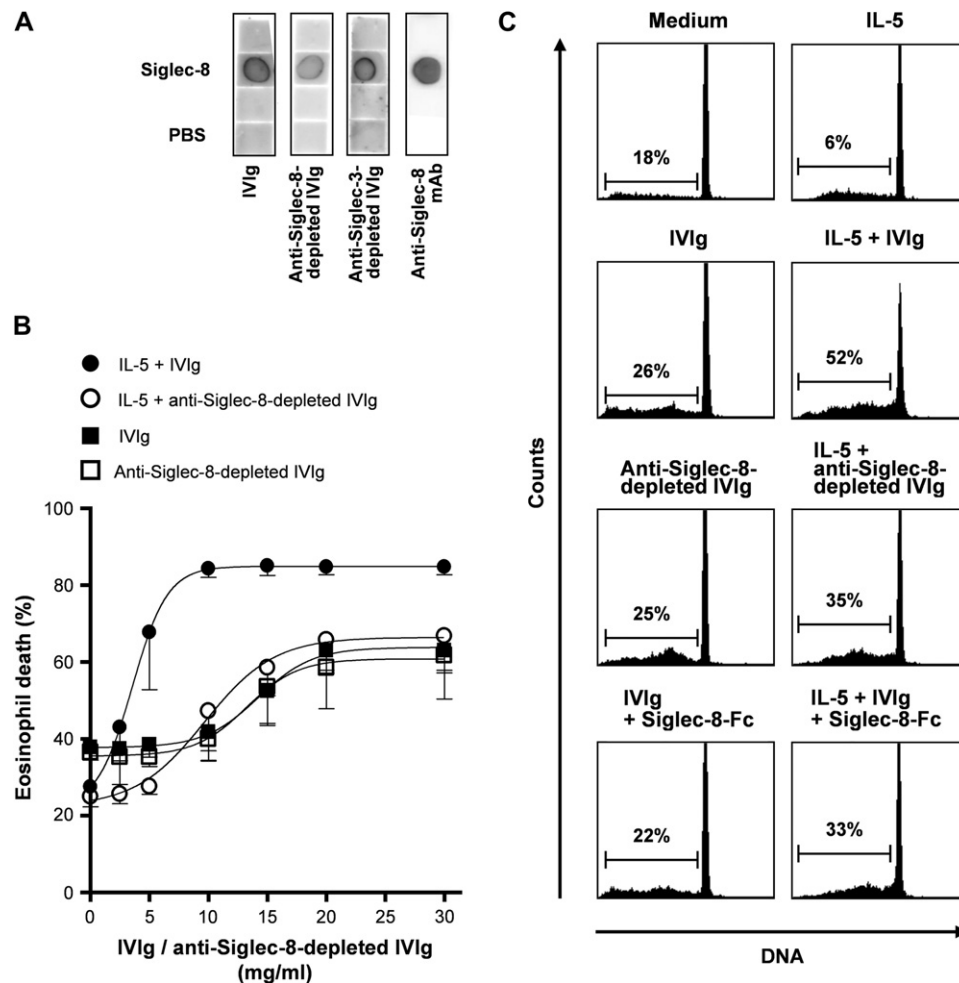


FIG 4. IVIg contains autoantibodies against human Siglec-8, which are responsible for the increased cytotoxicity of IVIg in the presence of IL-5. **A**, Dot blots of the binding activity of different fractions of IVIg (all at 10 μ g/mL) and a control anti-Siglec-8 mAb to immobilized recombinant Siglec-8/GST fusion protein are shown. The data are representative of 3 independent experiments. **B**, Concentration-effect curves of total IVIg and anti-Siglec-8-depleted IVIg, respectively, in 24-hour eosinophil cultures. In the absence of IL-5 pre-treatment, both death efficacy and potency of anti-Siglec-8-depleted IVIg was similar to total IVIg. Depletion of anti-Siglec-8 autoantibodies, however, resulted in loss of IL-5-mediated increase of death efficacy and potency of IVIg ($n = 4$). **C**, DNA fragmentation assay. Both IVIg and anti-Siglec-8-depleted IVIg induced DNA fragmentation in the absence of IL-5. In the presence of IL-5, IVIg treatment resulted in enhanced DNA fragmentation that was not observed if anti-Siglec-8-depleted IVIg was used. Similarly, pretreatment of total IVIg with Siglec-8/Fc fusion protein abolished the increased death effect of IVIg in the presence of IL-5. Results of 15-hour eosinophil cultures are shown that are representative of 3 independent experiments.

Fas receptor activation,⁵ are responsible for this phenomenon (Fig 4, C).

DISCUSSION

We provide evidence for the existence of autoantibodies directed against Siglec-8 in IVIg, which suggests that healthy persons have autoreactivity against this surface molecule that is expressed on eosinophils, basophils, and mast cells.⁷ Although a natural ligand of Siglec-8 has recently been identified,²³ it should be noted that the interaction between such autoantibodies and Siglec-8 may also occur under *in vivo* conditions.

Thus, anti-Siglec-8 autoantibodies might be considered as an additional physiologic ligand of Siglec-8 that stimulates Siglec-8 expressing cells. Previous work suggested that IVIg contains anti-Fas and anti-Siglec-9 autoantibodies.⁴⁻⁶ Such autoreactivity against membrane molecules on cells of the immune system is thought to play an important role in the anti-inflammatory action of IVIg.¹⁻³ Interestingly, the first evidence for the presence of both anti-Siglec-9⁶ and anti-Siglec-8 autoantibodies (shown in this study) was obtained in functional tests.

We observed that IVIg exhibited high caspase-independent eosinophil cytotoxicity in the presence of the eosinophil hematopoietins IL-5 and GM-CSF. A previous report demonstrated the induction of eosinophil death

after ligation of Siglec-8 with an anti-Siglec-8 mAb that was enhanced in the presence of IL-5 and GM-CSF.⁷ Based on the similarities between anti-Siglec-8- and IVIg-mediated eosinophil death, we hypothesized that IVIg contains natural anti-Siglec-8 autoantibodies. This hypothesis was further supported by the recent observation that anti-Siglec-9 autoantibodies are present in IVIg preparations.⁶ We therefore generated anti-Siglec-8-depleted IVIg to perform binding and functional studies in comparison with total IVIg. The data obtained using anti-Siglec-8-depleted IVIg suggested that the additional caspase-independent death mediated by total IVIg in the presence of IL-5 was largely caused by anti-Siglec-8 autoantibodies. Whether these antibodies may also exhibit a pathologic role remains to be determined. Clearly, a test suitable for the quantification of anti-Siglec-8 autoantibodies in single sera of patients would be helpful to address such a question in future studies.

The results of the current study have several implications for IVIg treatment. For instance, the increased efficacy and potency of IVIg in a cytokine-rich environment might explain the local anti-inflammatory effects of systemically applied IVIg. The IVIg effects observed in this report were observed between 1 and 10 mg/mL, which is a concentration range that is reached in blood under *in vivo* conditions.⁵ After diffusion from the intravascular compartment to the site of inflammation, the concentrations of IVIg are likely to be reduced but may still be sufficient to induce eosinophil death, which may limit eosinophil inflammatory responses. As IVIg has been reported to be beneficial in patients with Churg-Strauss syndrome,²⁴⁻²⁶ it is possible that the described cytotoxic effects on eosinophils result in reduced eosinophil numbers at the site of inflammation. This result would also explain, at least partially, why IVIg treatment often allows the reduction of the systemic steroid dose in Churg-Strauss syndrome and in other eosinophilic patients. Future work is required to develop biomarkers, which may include functional assays as described here, to identify those patients with severe eosinophilic inflammatory diseases that are likely to benefit from IVIg treatment.

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REFERENCES

- Kazatchkine MD, Kaveri SV. Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. *N Engl J Med* 2001;345:747-55.
- Simon HU, Späth PJ. IVIG—mechanisms of action. *Allergy* 2003;58:543-52.
- Jolles S, Sewell WA, Misbah SA. Clinical uses of intravenous immunoglobulin. *Clin Exp Immunol* 2005;142:1-11.
- Prasad NK, Papoff G, Zeuner A, Bonnin E, Kazatchkine MD, Ruberti G, et al. Therapeutic preparations of normal polyspecific IgG (IVIg) induce apoptosis in human lymphocytes and monocytes: a novel mechanism of action of IVIg involving the Fas apoptotic pathway. *J Immunol* 1998;161:3781-90.
- Altznauer F, von Gunten S, Späth P, Simon HU. Concurrent presence of agonistic and antagonistic anti-CD95 autoantibodies in intravenous Ig preparations. *J Allergy Clin Immunol* 2003;112:1185-90.
- von Gunten S, Schaub A, Vogel M, Stadler BM, Miescher S, Simon HU. Immunologic and functional evidence for anti-Siglec-9 autoantibodies in intravenous immunoglobulin preparations. *Blood* 2006;108:4255-9.
- Nutku E, Aizawa H, Hudson SA, Bochner BS. Ligation of Siglec-8: a selective mechanism for induction of human eosinophil apoptosis. *Blood* 2003;101:5014-20.
- Nutku E, Hudson SA, Bochner BS. Mechanism of Siglec-8-induced human eosinophil apoptosis: role of caspases and mitochondrial injury. *Biochem Biophys Res Commun* 2005;336:918-24.
- von Gunten S, Yousefi S, Seitz M, Jakob SM, Schaffner T, Seger R, et al. Siglec-9 transduces apoptotic and non-apoptotic death signals into neutrophils depending on the pro-inflammatory cytokine environment. *Blood* 2005;106:1423-31.
- Simon HU, Weber M, Becker E, Zilberman Y, Blaser K, Levi-Schaffer F. Eosinophils maintain their capacity to signal and release eosinophilic cationic protein upon repetitive stimulation with the same agonist. *J Immunol* 2000;165:4069-75.
- Simon HU, Plötz S, Simon D, Seitzer U, Braathen LR, Menz G, et al. Interleukin-2 primes eosinophil degranulation in hypereosinophilia and Wells' syndrome. *Eur J Immunol* 2003;33:834-9.
- Vassina EM, Yousefi S, Simon D, Zwicky C, Conus S, Simon HU. cIAP-2 and survivin contribute to cytokine-mediated delayed eosinophil apoptosis. *Eur J Immunol* 2006;36:1975-84.
- Floyd H, Ni J, Cornish A, Zeng Z, Liu D, Carter K, et al. Siglec-8. A novel eosinophil-specific member of the immunoglobulin superfamily. *J Biol Chem* 2000;275:861-6.
- Baumann R, Casaulta C, Simon D, Conus S, Yousefi S, Simon HU. Macrophage migration inhibitory factor delays apoptosis in neutrophils by inhibiting the mitochondria-dependent death pathway. *FASEB J* 2003;17:2221-30.
- Yousefi S, Hoessli D, Blaser K, Mills G, Simon HU. Requirement of Lyn and Syk tyrosine kinases for the prevention of apoptosis by cytokines in human eosinophils. *J Exp Med* 1996;183:1407-14.
- Yousefi S, Perozzo R, Schmid I, Ziemiecki A, Schaffner T, Scapozza L, et al. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. *Nature Cell Biol* 2006;8:1124-32.
- Altznauer F, Martinelli S, Yousefi S, Thürlig C, Schmid I, Conway EM, et al. Inflammation-associated cell cycle-independent block of apoptosis by survivin in terminally differentiated neutrophils. *J Exp Med* 2004;199:1343-54.
- Conus S, Bruno A, Simon HU. Leptin is an eosinophil survival factor. *J Allergy Clin Immunol* 2005;116:1228-34.
- Plötz SG, Simon HU, Darsow U, Simon D, Vassina E, Yousefi S, et al. Use of an anti-interleukin-5 antibody in the hypereosinophilic syndrome with eosinophilic dermatitis. *N Engl J Med* 2003;349:2334-9.
- Simon HU. Molecules involved in the regulation of eosinophil apoptosis. *Chem Immunol Allergy* 2006;91:49-58.
- Simon HU, Haj-Yehia A, Levi-Schaffer F. Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis* 2000;5:415-8.
- Mollnes T, Brekke O, Fung M, Fure H, Christiansen D, Bergseth G, et al. Essential role of the C5a receptor in E coli-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation. *Blood* 2002;100:1869-77.
- Bochner BS, Alvarez RA, Mehta P, Bovin NV, Blixt O, White JR, et al. Glycan array screening reveals a candidate ligand for Siglec-8. *J Biol Chem* 2005;280:4307-12.
- Klion A, Bochner BS, Gleich G, Nutman T, Rothenberg M, Simon HU, et al. Approaches to the treatment of hypereosinophilic syndromes: a workshop summary report. *J Allergy Clin Immunol* 2006;117:1292-302.
- Takigawa N, Kawata N, Shibayama T, Tada A, Kimura G, Munemasa M, et al. Successful treatment of a patient with severe Churg-Strauss syndrome by a combination of pulse corticosteroids, pulse cyclophosphamide, and high-dose intravenous immunoglobulin. *J Asthma* 2005;42:639-41.
- Tsurikawa N, Taniguchi M, Saito H, Himeno H, Ishibashi A, Suzuki S, et al. Treatment of Churg-Strauss syndrome with high-dose intravenous immunoglobulin. *Ann Allergy Asthma Immunol* 2004;92:80-7.