Intravenous immunoglobulin (IVIg) inhibits CD8 cytotoxic T-cell activation

We recently reported that intravenous immunoglobulin (IVIg) interferes with the binding of ovalbumin immune complexes (OVA-IC) to phagocytic FcγRs, leading to a decreased internalization inside antigen-presenting cells (APCs) and resulting in a reduced amount of antigen presented by MHC II molecules to CD4 helper T cells.1 Consequently, the antigen-specific helper T-cell response is dampened in the presence of IVIg. In the context of autoimmune diseases, these observations suggest that IVIg treatment could decrease the presentation of self-antigens to CD4 T cells and prevent the subsequent autoantibody production.

Although CD8 cytotoxic T cells play a substantial role in organ destruction in several autoimmune diseases,2,3 the effect of IVIg on this cell compartment has not been studied so far. We hypothesized that CD8 T-cell activation could be impaired in the presence of IVIg, by a mechanism similar to that described for CD4 T-cell activation. We thus used OT-II (CD4) I-Ab–restricted OVA–specific primary T cells5 to first confirm that the previously reported inhibitory effect of IVIg was not limited to I-Ad–restricted OVA–specific CD4 T cells (DO-11.10).1 We also used OT-I (CD8) H2Kb–restricted OVA–specific T cells6 to determine the effect of IVIg on the ability of APCs to activate CD8 T cells by cross-presentation of OVA-IC. OT-I and OT-II cells were purified from the spleen and lymph nodes of C57BL/6-Tg(TcraTcrb)425Cbn/J mice, respectively. Bone marrow–derived dendritic cells (BMDCs) from C57BL/6 mice were prepared as previously described and used as APCs to activate OT-I and OT-II cells in the presence of OVA-IC, with or without IVIg. T-cell activation was determined by flow cytometry using CD69 expression as a marker of cell activation.7

Our results first show an increased proportion of OT-II cells expressing CD69 after OVA-IC presentation, from a background level of 3% up to 74% (Figure 1 top left and middle panels). When IVIg was present during OVA-IC presentation, the percentage of cells expressing CD69 only reached 10% (Figure 1 top right panel), indicating that OT-II cell activation was significantly impaired in the presence of IVIg. The inhibitory effect of IVIg on OT-II cell activation is thus similar to that previously observed with DO-11.10 cells, regardless of their different MHC restriction profile. Our results also reveal the efficient activation of OT-I cells by OVA-IC cross-presentation, as shown by the increase from a background level of 1% to a proportion of 29% of C69-expressing cells (Figure 1 bottom left and middle panels). However, the presence of IVIg completely prevented OT-I cell activation, as illustrated by the absence of induction of CD69 on these cells (bottom right panel).

The effect of IVIg on CD8 T-cell activation by cross-presentation of immune complexes was predictable, because CD8 T-cell activation requires signals provided by antigenic epitopes presented on MHC I molecules.8,9 We herein provide the experimental demonstration of this inhibitory effect. Whether the interference of IVIg with immune complex uptake by APCs is solely responsible for this inhibition remains to be determined. Kaveri et al previously showed that IVIg contains antibodies specific to a highly conserved portion of human HLA class I antigens and that these antibodies were able to inhibit class I–restricted T cell–mediated cytotoxicity.10 However, we did not observe MHC I blockade by IVIg in our assays, suggesting that HLA class

References
I–specific antibodies are not involved in the inhibitory effects reported here.

In conclusion, we demonstrated that antigen-specific CD8 T-cell activation after cross-presentation of immune complexes by BMDCs is strongly reduced in the presence of therapeutic doses of IVIg. This observation extends our previous observations showing that antigen-specific CD4 T-cell activation is inhibited by IVIg both in vitro and in vivo. Altogether, these results suggest that not only CD4 but also CD8 T-cell activation should be considered as therapeutic targets in the development of potent substitutes to IVIg.

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References


To the editor:

Lack of association between KIR genes and acute lymphoblastic leukemia in children

In a recent report, Almalte et al described novel associations between childhood acute lymphoblastic leukemia (ALL) and killer immunoglobulin-like receptor (KIR) genes in a case-control study including mostly French-Canadian patients. The study was limited to the analysis of stimulatory KIR (KIR-S) and impressively, all of the 6 different KIR-S exhibited a strongly reduced frequency in the patient cohort. We performed a similar analysis in a cohort of childhood B-ALL (n = 185) and T-ALL (n = 33) patients of European origin (92% German, recruitment 1992-2012) from the pediatric oncology center in Düsseldorf, but also included inhibitory KIR, which enabled the identification of extended KIR haplotypes. As shown in Figure 1A, none of the KIR-S genes exhibited a significant frequency deviation from our ethnically matched control cohort. Our control group exhibited comparable KIR-S frequencies to the French-Canadian control group from Almalte et al except for KIR2DS5, which was unusually high in the Canadian study also when compared with other white cohorts from France, Germany, or the United Kingdom (data available at www.allelefrequencies.net). Because the strongest association in that study was seen for KIR2DS2, we looked for the frequency of the inhibitory KIR2DL2, which is in strong linkage disequilibrium with KIR2DS2. Again no decreased frequency of KIR2DL2 was found in our ALL cohort. The data from Almalte et al also implicate that the frequency of group A KIR haplotypes, which are abundant in white populations and harbor only a single KIR-S, would be much higher in ALL patients. Again our analysis does not show any significant difference between patients and controls (Figure 1B).

Further analysis of telomeric and centromeric KIR haplotypes as well as the cumulative number of stimulatory KIR genes did not reveal any significant difference to the control cohort (data not shown).

Given the technical challenges associated with PCR-based KIR genotyping, which is due to the strong similarity between KIR genes and the increasing number of alleles, it is generally helpful to assess extended KIR haplotypes when performing case-control studies. Because of the strong linkage disequilibrium between several pairs of KIR, the knowledge of KIR haplotypes provides an important plausibility control for KIR typing results. Moreover, in our experience historic patient sample collections can be particularly challenging for KIR typing, leading to decreased amplification efficiency compared with high-quality control samples. Given the consistently decreased frequencies of all KIR-S genes in the Almalte et al study, it would be highly desirable to know inhibitory KIR gene frequencies in this cohort, which would help to understand how the distribution of KIR haplotypes is affected. Unfortunately, PCR primers and amplification conditions used for KIR
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