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References

1. Verstovsek S, Mesa RA, Gotlib J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med.* 2012;366(9):799-807.
2. Harrison C, Kiladjian JJ, Al-Ali HK, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N Engl J Med.* 2012;366(9):787-798.
3. Verstovsek S, Kantarjian HM, Estrov Z, et al. Long-term outcomes of 107 patients with myelofibrosis receiving JAK1/JAK2 inhibitor ruxolitinib: survival advantage in comparison to matched historical controls. *Blood.* 2012;120(6):1202-1209.

To the editor:

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,[2-4] 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 cells[5] 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) $H2K^b$-restricted OVA-specific T cells[6] 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)1100Mjb/J and B6.Cg-Tg(TcraTcrb)425Cbn/J mice, respectively. Bone marrow–derived dendritic cells (BMDCs) from C57BL/6 mice were prepared as previously described[1] 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).

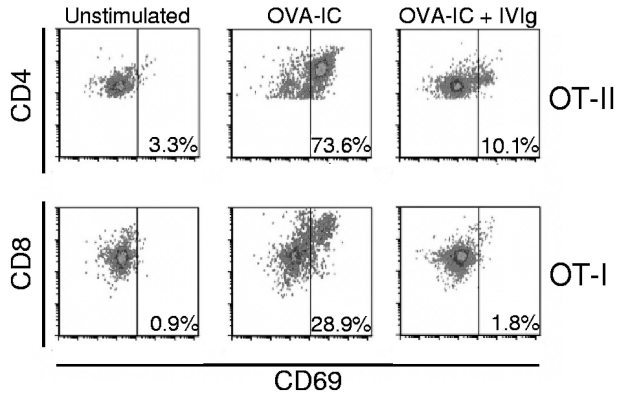

Figure 1. Prevention of OVA-IC–mediated OT-II (CD4) and OT-I (CD8) activation by IVIg. Equal numbers of bone marrow–derived dendritic cells (BMDCs) and OT-II (top panels) or OT-I (bottom panels) cells were incubated for 3 days in the presence of 2.5 μg/mL of OVA-IC prepared as described previously,[1] with or without 10 mg/mL of IVIg (Gamunex). The background activation was established in the absence of OVA-IC (left panels). After incubation, the cells were recovered, washed and labeled with either anti–CD4-FITC or anti–CD8-PE and anti–CD69-APC (eBioscience). T-cell activation was determined by measuring the expression of CD69 on CD4- (top panels) or CD8- (bottom panels) gated cells by flow cytometry (Accuri C6 flow cytometer). The data were analyzed using the FCS Express 4.0 software (De Novo) and are representative of 4 independent experiments done with different lots of BMDCs.

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

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

1. Aubin E, Lemieux R, Bazin R. Indirect inhibition of in vivo and in vitro T-cell responses by intravenous immunoglobulins due to impaired antigen presentation. *Blood.* 2010;115(9):1727-1734.
2. Tsai S, Shameli A, Santamaria P. CD8+ T cells in type 1 diabetes. *Adv Immunol.* 2008;100:79-124.
3. Friese MA, Fugger L. Autoreactive CD8+ T cells in multiple sclerosis: a new target for therapy? *Brain.* 2005;128(Pt 8):1747-1763.
4. Liblau RS, Wong FS, Mars LT, Santamaria P. Autoreactive CD8 T cells in organ-specific autoimmunity: emerging targets for therapeutic intervention. *Immunity.* 2002;17(1):1-6.
5. Barnden MJ, Allison J, Heath WR, Carbone FR. Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements. *Immunol Cell Biol.* 1998;76(1):34-40.
6. Hogquist KA, Jameson SC, Heath WR, Howard JL, Bevan MJ, Carbone FR. T cell receptor antagonist peptides induce positive selection. *Cell.* 1994;76(1):17-27.
7. Ziegler SF, Ramsdell F, Alderson MR. The activation antigen CD69. *Stem Cells.* 1994;12(5):456-465.
8. Zhang N, Bevan MJ. CD8(+) T cells: foot soldiers of the immune system. *Immunity.* 2011;35(2):161-168.
9. Prlic M, Williams MA, Bevan MJ. Requirements for CD8 T-cell priming, memory generation and maintenance. *Curr Opin Immunol.* 2007;19(3):315-319.
10. Kaveri S, Vassilev T, Hurez V, et al. Antibodies to a conserved region of HLA class I molecules, capable of modulating CD8 T cell-mediated function, are present in pooled normal immunoglobulin for therapeutic use. *J Clin Invest.* 1996;97(3):865-869.

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.[1] 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* genotypes. 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[1] 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[2] 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* genotypes when performing case-control studies. Because of the strong linkage disequilibrium between several pairs of *KIR*, the knowledge of *KIR* genotypes 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,[1] it would be highly desirable to know inhibitory *KIR* gene frequencies in this cohort, which would help to understand how the distribution of *KIR* genotypes is affected. Unfortunately, PCR primers and amplification conditions used for KIR