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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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

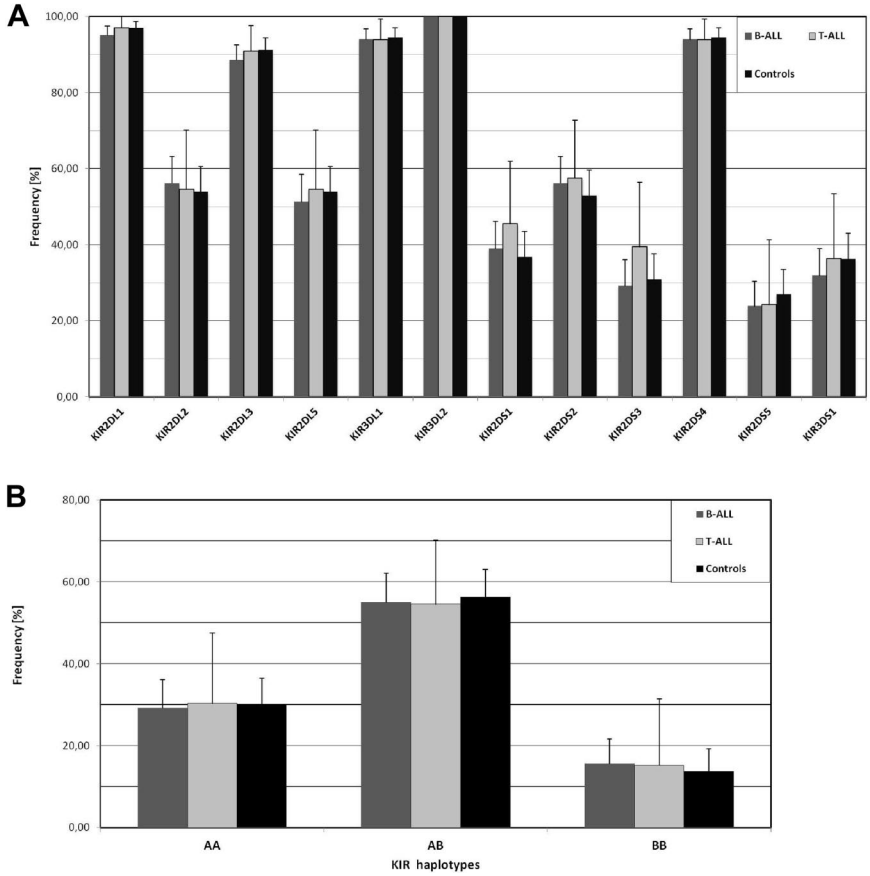

Figure 1. No association of childhood ALL with *KIR* gene frequencies. (A) The frequency of 6 inhibitory *KIR* genes (*KIR2DL1, 2DL2, 2DL3, 2DL5, 3DL1, 3DL2*) and 6 stimulatory *KIR* genes (*KIR2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DS1*) was analyzed in ALL patients. Our study population (0-18 years) consisted of 185 children with B-ALL (dark gray columns) and 33 children with T-ALL (light gray columns) of European origin. The ethnically matched control group (black columns) consisted of 204 unrelated randomly selected healthy volunteers. PCR-based *KIR* genotyping was performed as described by Vilches et al.[3] As an additional quality control, 10% of samples were randomly selected and analysis repeated with an independent *KIR* typing protocol as described by Uhrberg et al.[4] Similarly, all samples exhibiting rare *KIR* genotypes (frequency $< 0.5\%$) were controlled in this way.[4] Samples with discordant typing results ($n = 9$) were excluded from the analysis. (B) Distribution of group *A* and *B KIR* haplotypes according to previous definitions.[5] Statistical significance was tested by 2-sided Student *t* test and 95% confidence intervals are indicated.

typing were not specified. The lack of these data in the study by Almalte et al makes it difficult to assess the observed discrepancies between the 2 studies.

In summary, we could not confirm the association of *KIR-S* genes with the risk of childhood ALL in our cohort and would generally recommend the assessment of extended *KIR* genotypes when performing case-control studies.

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Response

Associations between activating *KIR* genes and childhood leukemia

We are surprised to know that Babor et al did not detect any significant differences in the frequencies of activating *KIR* genes between acute lymphoblastic leukemia (ALL) patients and healthy controls.[1] Obviously, these results contradict those reported recently by us.[2] Interestingly, the frequencies of these genes in controls are more or less comparable in both studies. Babor and colleagues mentioned technical challenges associated with PCR-based *KIR* genotyping due to strong similarities between *KIR* genes and their many alleles. They further note that because of the archived nature of patient samples, the quality of the DNA may be compromised and hence it may be difficult to amplify *KIR* genes. Therefore, the authors imply that this could be one reason for decreased *KIR* gene frequencies in our patient samples. We have experienced such difficulties in amplifying *KIR* genes in both patient and control samples with longer amplifications. Such difficulties were alleviated when smaller segments of the genes were amplified. All of our PCR reactions for the *KIR* genes amplified $\leq$ 300 bp bands. Furthermore, we have used the same genotyping methods to determine gene frequencies of activating *KIR* genes in other diseases (eg, Crohn disease) and have found increased frequencies of several of the activating *KIR* genes in the patients compared with the controls (data not shown). We receive samples from both patients and controls for these studies from DNA banks. Furthermore, all of the patient and control DNA samples yielded bands in positive control reactions. Therefore, we do not believe that the decreased frequencies of activating *KIR* genes in leukemia patients are due to inherent unsuitability of our DNA samples for PCR-based amplifications. After receiving an invitation to respond to the letter by Babor et al, we regenotyped a subset of our patients and controls. Again, we found significantly decreased frequencies of all activating *KIR* genes in our cohorts. It is noteworthy that decreased frequencies of some activating *KIR* genes in ALL patients have been described.[3,4]

The reasons for the discordant results in the 2 studies are not immediately apparent. However, we speculate that these differences may result from the use of different PCR primer sets. We would encourage Babor and colleagues to verify their results with our primer pairs. We can provide them or send them their exact sequences. We would also be willing to genotype our DNA samples (a subset or all) in our laboratory. Furthermore, it could also be possible that these associations may not be present in the German population. Literature is replete with examples where genetic associations with a disease in one population were not replicated in another.

Concerning inhibitory *KIR* genes in our ALL patients, we are in the process of genotyping them. It is premature to draw definitive conclusions. However, we can say with certainty that they are showing a trend opposite to that of activating genes. This trend also verifies the suitability of our DNA samples for PCR-based genotyping. Concerning KIR haplotypes, our results do suggest that B haplotypes reduce risk for ALL. However, we have not directly determined them in our patient and control samples.

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