Comparison of Three Methods for the Evaluation of Cytokine Storm Risk in Early- and Clinical- Stage Biopharmaceutical Development

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INTRODUCTION

Cytokine Release Syndrome and Cytokine Storm (CRS or CS) are potentially fatal immune reactions characterized by large-scale release, upon a first infusion of some therapeutic antibodies or biologics, of the proinflammatory cytokines IL2, IL4, IL6, IL8, IL17, TGFb, TNFa and/or IFNg by immune cells.

In 2006, a Phase I clinical trial of TGN1412, a humanized anti-CD28 superagonist, caused a near-fatal cytokine storm reaction in 6 out of 6 healthy volunteers. The adverse reaction to TGN1412 was not predicted by the standard battery of preclinical in vitro or in vivo tests, including NHP GLP studies. Subsequent analysis of interspecies differences found that the underlying cells responsible for the cytokine storm, CD4+CD45RO+ effector-memory T cells, express CD28 in humans but not in primates. In addition, human lymphoid T cells, but not peripheral blood effector-memory T cells, were found to be reactive to TGN1412 raising questions about the utility of in vitro or ex vivo testing using PBMCs for CRS.

Prior to the TGN1412 clinical trial disaster, the standard method for in vitro assessment of cytokine release syndrome was to add aqueous test antibody or biologic to a culture of PBMCs for 24 to 72 hours and then measure cell proliferation and inflammatory cytokine release. This simple approach is now known to lack sufficient sensitivity to accurately assess risk of CRS or CS. Since then, a great deal of effort has gone into creating an in vitro assay that is capable of recapitulating in vivo effects. At least three methods have been reported that improve, in some ways, the ability to predict CRS: (a) immobilization of test antibody on plastic, (b) co-culture of PBMCs on HUVECs and (c) pre-culture of PBMCs at high cell density.

OBJECTIVE

The goal of this work is to identify an assay that can accurately predict the risk of CRS and CS associated with investigational biotherapeutics. The assay must be suitable for routine use in a biotherapeutics development program and must use soluble, not plastic-immobilized, test article to allow for meaningful comparisons between test and control articles and correlation with in vivo dosing. To be considered predictive, the assay should, as a starting point, recapitulate in vitro the known in vivo biology of CRS in that subsaturating concentrations of anti-CD3 (clone OKT3) and saturating concentrations of superagonisitc anti-CD28 should each trigger pro-inflammatory cytokine production.

MATERIALS AND METHODS

Antibodies (anti-CD3, clone OKT3 and anti-CD28SA, clone ANC28.1) known to stimulate cytokine release and considered to be of clinical relevance were used as test articles. Humanized anti-CD20, which is not typically associated clinically with CRS or CS, was included as a negative control in all experiments. HUVECs (Lonza, MD) were cultured to confluence in 96 well round-bottom tc flasks using EBM-2 medium (Lonza) supplemented with 10% HI-FBS. Human leukapheresis-processed PBMCs were obtained from ReachBio. We compared three methods to identify a robust assay that allows for the assessment of potential CRS and CS risk. In Method 1 (Römer, 2011), human PBMCs were pre-incubated for 48 hrs at 1 x 10^7 cells per mL (high density), followed by culture on tissue-culture plastic in the presence of test (anti-CD3 clone OKT3 or anti-CD28 superagonist clone ANC28.1) or control (humanized anti-CD20 antibody or humanized anti-TNFα antibody) material. In Method 2 (Findlay, 2011), human PBMCs were cultured at 1 x 10^6 cells per mL on a HUVEC monolayer in the presence of test or control material. In Method 3 we used a combination of the first two methods whereby human PBMCs were pre-incubated at 1 x 10^7 cells per mL (high density), followed by culture at 1 x 10^6 cells per mL on a HUVEC monolayer in the presence of test or control material. In all studies, supernatants were harvested after 48 hours and evaluated for IL2, IL6, TNFα and IFNγ by ELISA.

FIGURE 1. Secretion of IFNg (measured by ELISA) using Method 2. Pro-inflammatory cytokine production is seen at saturating concentrations of anti-CD28SA and subsaturating concentrations of anti-CD3, but not anti-CD20.
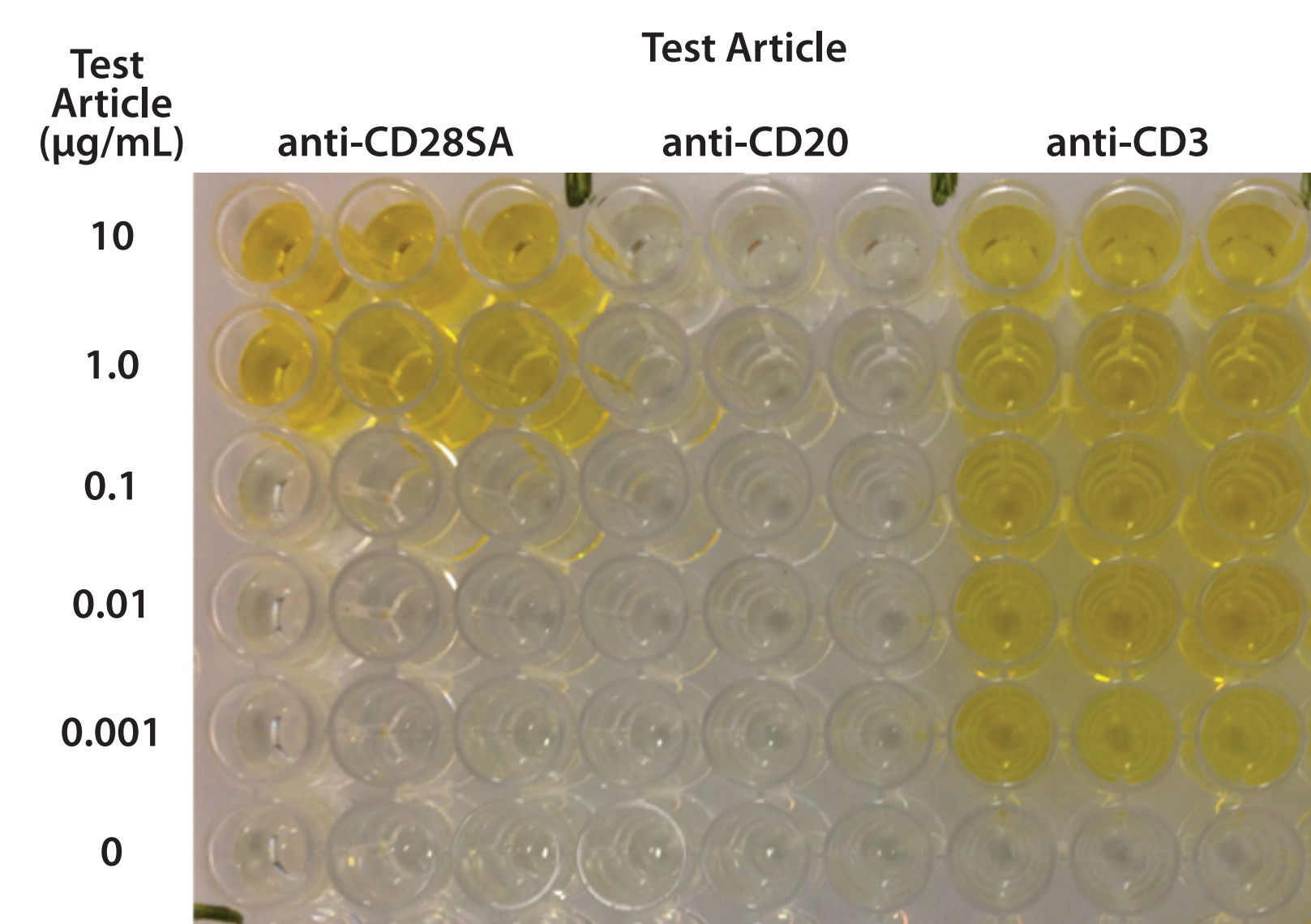

FIGURE 2. Pro-inflammatory cytokine production by NHP PBMCs and human PBMCs using Method 2. Figure 2A. Cytokine production after incubation with saturating amounts of anti-CD3. Figure 2B. Cytokine production after incubation with saturating amounts of anti-CD28SA.
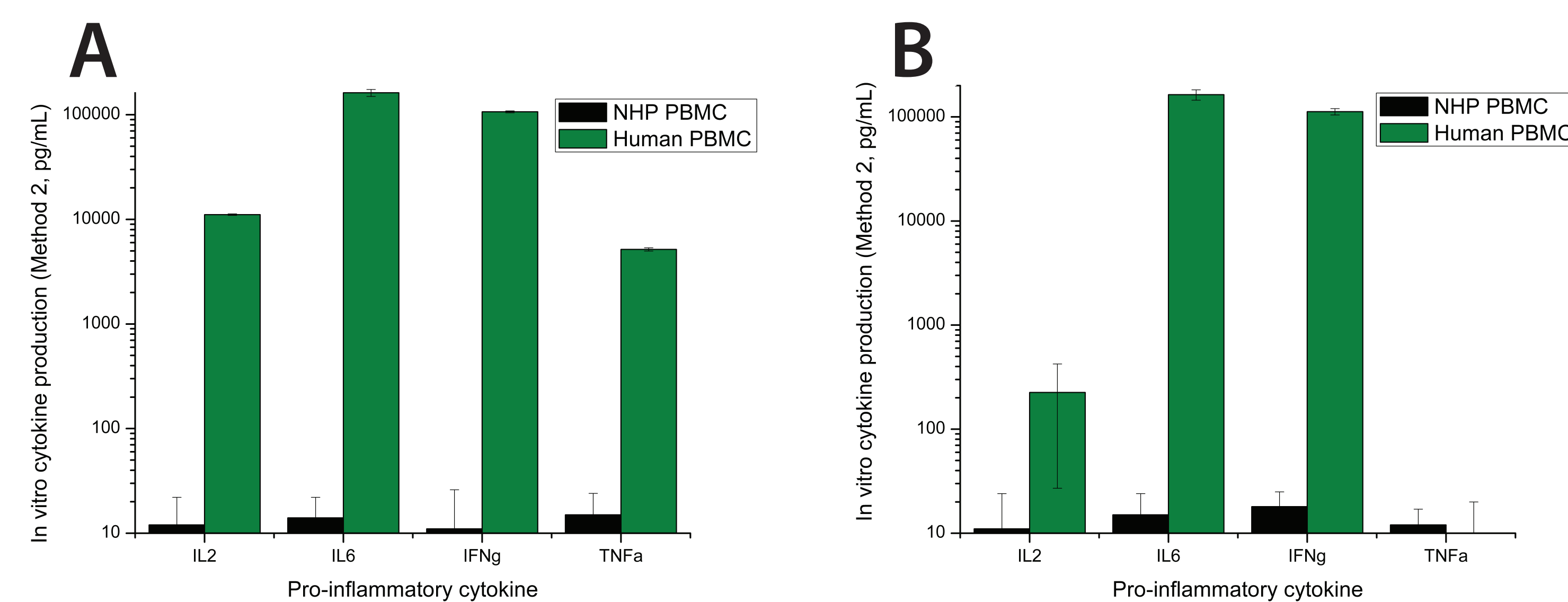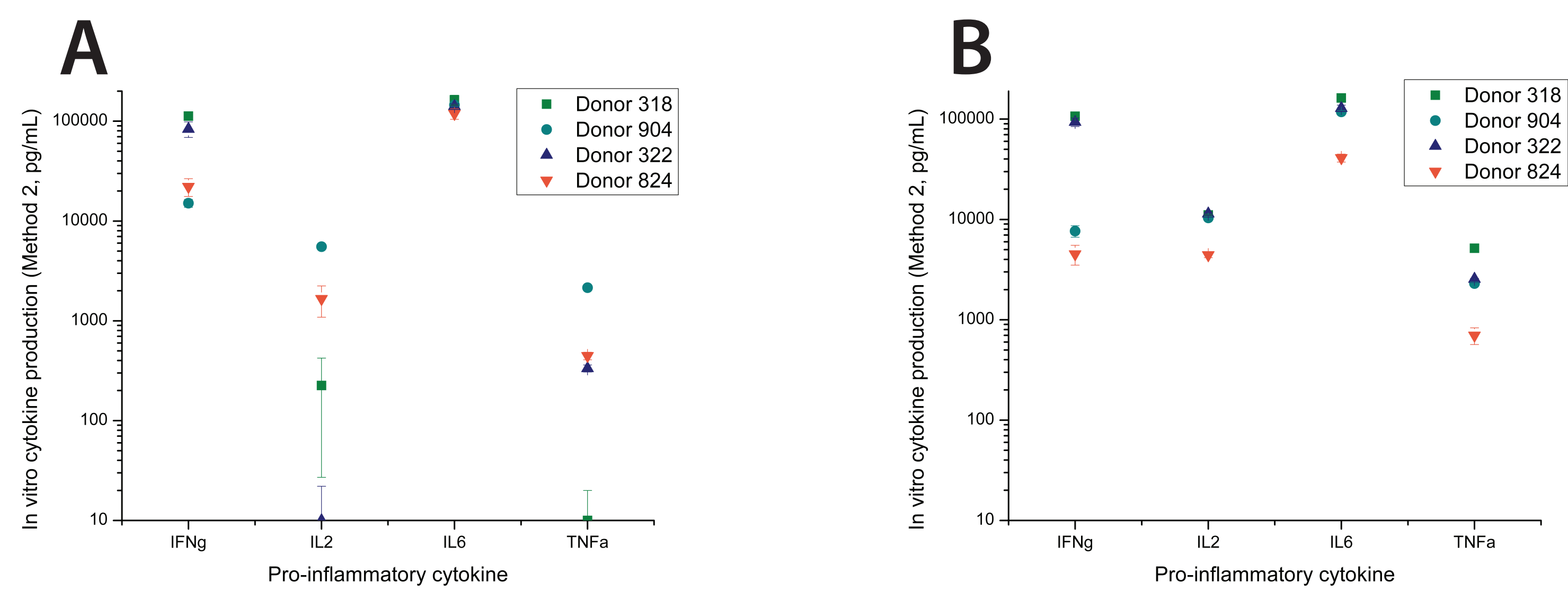

FIGURE 3. Pro-inflammatory cytokine production by four unique human PBMC donors using Method 2. Figure 3A. Cytokine production after incubation with saturating amounts of anti-CD3. Figure 3B. Cytokine production after incubation with saturating amounts of anti-CD28SA.

RESULTS

Pre-incubation of PBMCs at high density (Methods 1 and 3) resulted in low (<50%) PBMC viability when the test articles were introduced. Subsequently, no pro-inflammatory cytokine production was seen using Method 1 and relatively low amounts were seen using Method 3.

Method 2, in which human PBMCs were co-incubated on human HUVECs in the presence of test article, resulted in secretion of pro-inflammatory cytokines at subsaturating concentrations of soluble anti-CD3 and saturating concentrations of soluble anti-CD28SA. Humanized anti-CD20, which is not typically associated with clinical CRS or CS, did not trigger cytokine release under any of the conditions tested (Figure 1).

Fresh NHP and human PBMCs were compared using Method 2. Significant pro-inflammatory cytokine production was seen with human, but not NHP, PBMCs when challenged with either anti-CD3 or anti-CD28SA (Figures 2A and 2B).

To characterize donor variability using Method 2, a total of four PBMC donors were evaluated. All donors had similar pro-inflammatory cytokine release patterns at saturating concentrations of anti-CD28SA, but donor variability was observed when cells were exposed to anti-CD3 (Figures 3A and 3B).

CONCLUSIONS

Investigational therapeutics in their soluble (ie. not plastic-immobilized) form can be evaluated in vitro for their ability to trigger pro-inflammatory cytokine secretion by using a human primary cell HUVEC / PBMC co-culture system.

Clinically, anti-CD28SA was associated with severe CS in 6 out of 6 patients. Using Method 2 and saturating anti-CD28SA, we observed high concentrations (in excess of 1000 pg/mL) of all pro-inflammatory cytokines tested.

Using Method 2 and saturating anti-CD3, there was more donor variability of the four pro-inflammatory cytokines. Clinically, patient response to anti-CD3 therapy is also variable.

The method may have potential uses both at the development stage, to identify potential problems with an investigational biotherapeutic, and at the clinical stage, to identify at-risk patients prior to first infusion.

IL2 levels in excess of 1000 pg/mL may be a good benchmark for estimating risk of CRS or CS. Other pro-inflammatory cytokines should also be measured.

References

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