Different Preparations of Intravenous Immunoglobulin Vary in Their Efficacy to Neutralize Streptococcal Superantigens: Implications for Treatment of Streptococcal Toxic Shock Syndrome

Birgit Schrage, Guowen Duan, Lily P. Yang, John D. Fraser, and Thomas Proft

Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

Eight different batches of intravenous immunoglobulin from 3 different manufacturers were tested for neutralizing activities against all currently known streptococcal superantigens. Statistically significant variation among different intravenous immunoglobulin preparations \((P < .0001)\) and between individual streptococcal superantigens \((P < .0001)\) was observed. These results might be helpful for optimizing the type and dose of intravenous immunoglobulin used in adjunctive therapy for severe invasive streptococcal disease.

Since the 1980s, there has been a marked increase in highly invasive group A streptococcal (GAS) infection associated with shock and organ failure. These infections have been termed streptococcal toxic shock syndrome (STSS), with reported mortality rates of 30%–70% \([1]\). The involvement of multiple organs in STSS suggests that 1 or several toxins produced during GAS infection might be involved in pathogenesis. Prime candidates are the streptococcal superantigens, a family of highly mitogenic proteins that activate a large proportion of antigen-presenting cells and T cells, with subsequent release of high systemic levels of cytokines \([2]\). Circulating bioactive superantigens were found in the acute-phase serum samples of 2 patients with STSS \([3]\), and the lack of neutralizing antiserum antibodies appears to be a key risk factor for the development of staphylococcal and streptococcal toxic shock \([3, 4]\).

The results from several in vitro and in vivo studies strongly indicate that intravenous immunoglobulin (IVIG) therapy might be beneficial for treatment of STSS. Neutralization of superantigens is regarded as the major mode of action, but other effects have also been discussed \([5]\). IVIG has neutralized superantigen activity in cell culture supernatant from clinical GAS isolates, as well as recombinant streptococcal pyrogenic exotoxin (rSPE)–A and rSPE-C \([6]\). Since then, 10 additional streptococcal superantigens have been identified (reviewed in 2)).

For the first time, to our knowledge, this study analyzes the efficacy of toxin-neutralizing antibodies in commercially available IVIG preparations against the complete set of currently known streptococcal superantigens. The results might be useful in optimizing the type and/or dose of IVIG used for adjunctive therapy in severe invasive streptococcal disease.

**Materials and methods.** A total of 8 different IVIG samples from 3 manufacturers were analyzed in this study. They include 6 different batches of Intragram P (CSL), 1 batch of Vigam-S (BPL), and 1 batch of Endobulin S/D (Baxter AG). Intragram P was prepared from plasma collected by the Australian Red Cross from Australian volunteer donors and supplied to CSL. Vigam-S was prepared from plasma collected from donors in the United States, and Endobulin S/D was prepared from plasma collected from donors in Europe.

Recombinant forms of streptococcal superantigens were generated using the pGEX-3c expression system, as described elsewhere \([7]\). All toxins were used at 20 ng/mL in a standard peripheral blood lymphocyte proliferation assay in the presence or absence of various concentrations of IVIG. As a control, 5 \(\mu\)g/mL of phytohemagglutinin was used instead of superantigen. The peripheral blood lymphocytes were isolated from buffy coat preparations using Ficoll fractionation. All IVIG samples were supplemented with fetal calf serum (FCS) to a final IVIG/serum concentration of 10%. Stimulation of peripheral blood lymphocytes was expressed as counts per min (cpm). All samples were assayed in triplicate, and relative inhibition of superantigen activity was calculated as \(1 - [\text{cpm (IVIG)/cpm (FCS)}] \times 100\). To exclude potential nonspecific stimulation of T cells by IVIG, peripheral blood lymphocytes were incubated with individual IVIG preparations in the absence of superantigen. Statistically significant differences were assessed by an analysis of variance followed by the Tukey post hoc test and confirmed by logistic regression.

**Results.** Eight different batches of IVIG from 3 different manufacturers (CSL, BPL, and Baxter AG) were tested for the levels of neutralizing antibodies against recombinant forms of...
all currently known streptococcal superantigens: SPE-A, C, G, H, I, J, K/L, L/M, M, streptococcal mitogenic exotoxin Z (SMEZ)–1, SMEZ-2, and streptococcal superantigen (SSA). SPE-K/L is identical to SPE-K, which was identified by Beres et al. [8], and to SPE-L, which was identified by Ikebe et al. [9] and Proft et al. [10]. SPE-L/M is identical to SPE-L, which was identified by Smoot et al. [11], and to SPE-M, which was identified by Proft et al. [10]. SPE-M refers to SPE-M that was identified by Smoot et al. [11].

Each preparation of IVIG showed significant differences in neutralization efficacies against individual superantigens (P < 0.0001) (figure 1). The highest degree of neutralization for all IVIG samples was observed against SSA, SPE-A, SPE-C, and SPE-K/L, and SPE-J, SPE-H, and SMEZ-2 showed the lowest inhibition. Notably, none of the IVIG preparations completely inhibited SPE-J activity, even at 5 mg/mL. Differences in observed neutralization efficiency might be caused by a combination of antitoxin antibody titers, as well as by individual differences in toxin potencies. SMEZ-2 and SPE-J are very potent superantigens and are able to stimulate human T cells at much lower concentrations than, for example, SPE-A or SSA. On the other hand, SPE-C and SPE-J are equally potent, but SPE-C was neutralized far more efficiently. The mitogenic potencies for each of the superantigens, given as half maximum stimulation (P_{50}) values are: 0.02 pg/mL (SMEZ-2), 0.08 pg/mL (SMEZ-1), 0.1 pg/mL (SPE-C, SPE-I, and SPE-J), 1 pg/mL (SPE-K/L), 2 pg/mL (SPE-A and SPE-G), 10 pg/mL (SPE-L/M), and 50 pg/mL (SPE-H) [2]. The P_{50} value for SPE-M is not described in the literature.

The limited neutralization of SPE-H, which is the least potent of all streptococcal superantigens, might be explained by a very limited number of anti-SPE-H antibody specificities in the IVIG preparations. speH is a relatively rare streptococcal superantigen gene, which may explain the limited inhibitory activity of the pooled IVIG tested. A study of the frequency of sag genes among clinical GAS isolates showed that the speH gene was found in only 23 (24%) of 96 isolates collected in New Zealand, but smeZ was found in all isolates [7]. Other sag genes, such as speK/L, speL/M, and speM, are also rare, whereas speG, smeZ, and speI are commonly found in GAS isolates.

Notably, at higher dilutions of IVIG, an enhanced T cell response was consistently observed for SPE-J, SPE-H, and, to a lesser extent, SMEZ. Analysis for seroconversion in a patient with STSS also showed enhanced T cell proliferation at lower serum concentrations, and this was unique to SPE-J [3]. The basis for this enhanced activity is uncertain but could arise from unique antibodies that bind superantigens in such a way as to assist their presentation to T cells (in the absence of diluted inhibitory antibodies), either as dimeric soluble antibody/superantigen complex or attached to the antigen presenting cell surface via Fc receptor.

Our results reveal statistically significant differences in neutralizing activities among IVIG preparations from different manufacturers (P < 0.0001). Vigam-S, which is obtained from plasma collected from donors in the United States, showed consistently high inhibition against all superantigens. In contrast, Endobulin S/D, a European product, showed the lowest activity. The 6 different batches of Intragam P showed an inhibitory effect that was higher than Endobulin S/D and lower than Vigam-S, with only minor variations between individual batches. Intragam P is prepared from pooled plasma samples from Australian donors. The variations between IVIG preparations from different manufacturers are most likely caused by the different geographical regions from which the plasma samples were collected and might reflect differences in GAS exposure. This is consistent with a study by Norrby-Teglund et al. [6] that showed higher neutralization activity against SPE-A and SPE-C in an IVIG preparation from the United States (Venoglobulin-S Alpha Therapeutic), compared with IVIG preparations from Europe and Canada. Furthermore, a recent study of blood samples collected from communities of Pacific Islanders in South Auckland, New Zealand, and Aboriginal Australians from the Northern Territory who commonly have high GAS infection rates had significantly higher serum titers of neutralizing antibodies against all known streptococcal superantigens, compared with samples collected from persons residing in other communities with recognized lower rates of GAS exposure [12].

**Discussion.** Patients with STSS usually receive a single dose of 2 g/kg of IVIG. Although most superantigens should be neutralized by IVIG treatment, the results of this study showed the limited efficacy of IVIG in neutralizing the activity of certain toxins, in particular, SPE-J and SMEZ. These are potent toxins that have recently been linked to cases of STSS [3, 13].

Unfortunately, very little is known about the regulation of superantigen gene expression. In cell culture supernatants from various GAS isolates, it was found that SPE-A was produced at 0.03–16 μg/mL [14], SPE-C was produced at 0.9–12 μg/mL [14], and SPE-J was produced at 0 to >10 ng/mL [3], whereas SMEZ was produced at <1 ng/mL [3]. However, several recent studies suggest that superantigens are significantly upregulated during infection (reviewed in [2]). More recent results based on DNA microarray analysis and real-time PCR showed a robust increase of transcription levels for speA and speJ, when the GAS isolate was grown in human whole blood instead of medium [15]. Furthermore, speA, speJ, and smeZ transcription was strongly upregulated in a primate infection model. Notably, smeZ transcription levels were found to be 24 times higher than speA levels [16]. Regulation of other superantigens has not been studied yet, but their expression might also be upregulated during infection. Hence, it seems likely that, during infection,
Figure 1. A total of 8 different preparations of intravenous immunoglobulin (IVIG) from 3 different manufacturers (Intragam P [CSL], Vigam-S [BPL], and Endobulin S/D [Baxter AG]) were compared for neutralizing activity against streptococcal superantigens. The different preparations have varying levels of neutralizing activity against streptococcal superantigens. For a detailed description of the methods used, see the Methods section. The number of different blood donors used for the neutralization analysis is given in parentheses. SMEZ, streptococcal mitogenic exotoxin Z; SPE, streptococcal pyrogenic exotoxin; SSA, streptococcal superantigen.
many superantigens reach levels similar to or higher than the 20 ng/mL used in this study.

Notably, Endobulin S/D showed significantly lower inhibitory activity than IVIG preparations from the other 2 suppliers. Perhaps simple prescreening of IVIG batches to identify optimal batches and doses would improve the results of IVIG treatment, not only for STSS and severe invasive GAS infection, but also for other toxin-mediated diseases. It is entirely consistent that IVIG obtained from communities with greater exposure to streptococcal infection would yield, on average, serum with the most abundant and broadest repertoire of neutralizing antitoxin antibodies. For therapeutic purposes, where IVIG might be considered as a treatment for superantigen-mediated STSS, it would seem entirely prudent to first select a commercial IVIG with the highest level and broadest spectrum of neutralizing antibodies.

Acknowledgments

We would like to thank Dr. Bill Abbott for his valuable help with statistical analysis.

Financial support. Sir Charles Hercus Research Fellowship from the Health Research Council New Zealand (to T.P.). The Intragram samples were kindly provided by the Immunology Day Stay Unit at Auckland City Hospital (Auckland, New Zealand) and by the Clinical Haematology Department at North Shore Hospital (North Shore City, New Zealand). The Endobulin S/D and the Vigam S preparations were kindly provided by Dr. Shiranee Sriskandan (Imperial College, London, UK).

Potential conflicts of interest. All authors: no conflicts.

References