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BRIEF REPORT

Correlation Between Immunoglobulin Dose Administered and Plasma Neutralization of Streptococcal Superantigens in Patients With Necrotizing Soft Tissue Infections

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Analyses of plasma collected pre- and postadministration of intravenous immunoglobulin (IVIG) from patients with group A Streptococcus necrotizing soft tissue infections demonstrated a negative correlation between IVIG dose and toxin-triggered T-cell proliferation ($r = -.67$, $P < .0001$). One 25-g IVIG dose was sufficient to yield plasma-neutralizing activity against streptococcal superantigens.

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The effect of polyspecific intravenous immunoglobulin G (IVIG) was recently assessed in the INSTINCT study; a randomized, placebo-controlled trial of patients with necrotizing soft tissue infections (NSTIs) [1]. The results showed no differences in the primary outcome—that is, the physical component Short Form 36 (SF-36) score—between the placebo- and IVIG-treated groups. However, a favorable trend was noted in the subgroup of patients with NSTIs of head/neck/extremities, which are associated with a higher rate of group A streptococcal (GAS) or staphylococcal infections. This is in line with the recent meta-analysis of IVIG therapy in clindamycin-treated streptococcal toxic shock syndrome (STSS) in which adjunctive IVIG was associated with a survival benefit [2]. Mechanistic actions of IVIG in this setting include neutralization of GAS superantigens and dampening of inflammation [3–7].

There are unresolved issues regarding IVIG treatment in severe bacterial infections, including the optimal dosage. In the INSTINCT trial, a fixed dose of 25 g/day for 3 days was used, which is lower than the dosages used in previous reports on IVIG therapy in STSS [8–10]. Also, 1 dose of IVIG was allowed prior to randomization in INSTINCT, and 20 (40%) versus 8 (16%) of the patients in the placebo and IVIG groups, respectively, had received IVIG before inclusion in the trial. This raises the following questions: (1) What level of streptococcal superantigen neutralization is achieved in NSTI patients receiving 25 g IVIG for 3 days and (2) Did one 25-g dose of IVIG prior to randomization affect the neutralizing activity in plasma? Here, we addressed these questions by analyses of the patients' plasma.

METHODS

Ethical Considerations

The studies were approved by the Danish Medicines Agency as well as the regional ethics committee in Copenhagen, Denmark (ref. 1211709); Gothenburg, Sweden (ref. 930-12); Bergen, Norway (REC West; ref. 325786); and Stockholm, Sweden (ref. 2012/2110-31/2; 2006/229-31/3,2016/1415-32). The project was conducted in accordance with the Helsinki Declaration, and informed consent was obtained from all patients and healthy blood donors.

Patient Samples

Patient inclusion is outlined in Supplementary Figure 1. All patients were enrolled in the INFECT project [11] (ClinicalTrials.gov, NCT01790698), a multicenter, prospective observational cohort study on NSTIs. Some patients were co-enrolled in the INSTINCT trial [1] (ClinicalTrials.gov, NCT02111161). From the INSTINCT cohort, we selected patients infected with monomicrobial GAS for analyses (N = 19; 5 placebo- and 14 IVIG-treated). Due to the limited number of patients with GAS NSTIs treated with placebo, a comparator cohort was identified from the INFECT cohort including patients with monomicrobial GAS infection, who had not been treated with IVIG prior to inclusion, and with 2 or more plasma samples available. From these patients with GAS NSTIs, we identified

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matched groups (clindamycin, septic shock, and renal replacement treatment) of non–IVIG- or IVIG-treated patients (median total dose of IVIG, 75 g; range, 25–135 g) (Supplementary Figure 1). Plasma was sampled on indicated days using the standard operating procedure of the INFECT project.

Bacterial Strains

A panel of GAS strains from the INFECT cohort (N = 29) was used to prepare superantigen-containing supernatants from stationary-phase cultures as detailed in Darenberg et al [8].

Proliferation Assay

The supernatants were tested for superantigenic activity and neutralization by IVIG in a proliferation assay essentially as previously detailed [8]. In brief, peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of healthy volunteers and stimulated with a 1:100 dilution of GAS supernatants or as positive control with 5 µg/mL phytohemagglutinin-L (PHA; Sigma-Aldrich) in the presence of either 10% fetal calf serum (FCS) or 1% patient plasma supplemented with 9% FCS. IVIG (Privigen; CSL Behring) at different concentrations was also tested. [^{3}H]-Thymidine (PerkinElmer) uptake was assessed after 72 hours.

Statistical Analyses

We used the nonparametric Spearman's rank correlation coefficient test and Wilcoxon's signed rank test (Prism 7.0a software; GraphPad, Inc). *P* values less than .05 were considered statistically significant.

RESULTS

IVIG Neutralizes the Superantigenic Activity of GAS NSTI Strains

The IVIG preparation used in the INSTINCT trial was tested at different concentrations for its ability to neutralize superantigenic activity expressed by a panel of GAS NSTI strains of different serotypes and with varying superantigen gene profile (Supplementary Figure 2*A*). A dose-dependent neutralization of the bacterial supernatants was observed (Supplementary Figure 2*B*). Although there was variation between different GAS supernatants, all were efficiently (>90%) neutralized. For the screening of neutralizing activity in patient plasma, 2 GAS isolates were selected, including the 2 *emm* types that are overrepresented among severe invasive GAS infections (ie, *emm*1 [strain 2006] and *emm*3 [strain 5013]). Combined, the 2 strains covered a broad spectrum of superantigen genes, including streptococcal pyrogenic exotoxin A (*spe*A), *spe*J, streptococcal superantigen (*ssa*), and streptococccal mitogenic exotoxin Z (*smeZ*) (Supplementary Figure 2*A*). There was no significant difference in mitogenic activity elicited by 5013 and 2006 culture supernatants (Supplementary Figure 2*C*), but 5013 was more susceptible to IVIG neutralization (Supplementary Figure 2*B* and *D*).

Plasma Neutralizing Activity Correlates With the IVIG Dose Administered

Daily plasma samples collected from the 19 INSTINCT patients were tested in a proliferation assay using supernatants from the 2 selected GAS isolates. For comparison, plasma collected at days 0 and 3 from IVIG-treated or non–IVIG-treated INFECT patients (N = 32) were tested. The GAS supernatants generally elicited high PBMC proliferation in the presence of plasma collected days 0–3 from non–IVIG-treated patients (Figure 1A and B). In contrast, in IVIG-treated patients, a significant decline in proliferation was noted in the presence of posttreatment samples (Figure 1C and D). A negative dose–response was observed between IVIG and proliferation (strain 2006: $r = -.65$, $P < .0001$; strain 5013: $r = -.67$, $P < .0001$) (Figure 1E). Administration of 25 g of IVIG resulted in efficient plasma neutralization of the superantigen response to GAS 5013, but less so in strain 2006 (Figure 1C), consistent with the noted difference in IVIG-neutralization of the two strains (Supplementary Figure 2*D*).

DISCUSSION

In this follow up-study of the INSTINCT trial [1], we show that, in most cases, 25 g IVIG was sufficient to achieve plasma neutralization of GAS superantigenic activity. However, there were variations among GAS strains; for some strains, a repeat dose of 25 g was required to achieve a significant reduction in superantigenic responses. Further, a correlation between dose of IVIG administered and plasma-neutralizing activity of GAS superantigens was demonstrated.

This study focuses on patients with GAS-infected NSTIs, as the INSTINCT trial indicated a potential benefit of IVIG in this patient subgroup. As acknowledged in Madsen et al [1], the INSTINCT trial used a lower dose than in previous reports suggesting a beneficial effect of IVIG in STSS [8–10]. To test whether this lower dose of IVIG is sufficient to gain superantigen-neutralizing activity, we analyzed plasma collected pre– and post–study-drug administration. Our results demonstrated that plasma collected after 1 dose of 25 g IVIG resulted in a reduced superantigen response. These results may be of importance considering the INSTINCT trial protocol of allowing 1 dose of IVIG prior to study enrollment, especially as there was an uneven distribution, with 40% of the placebo group having received 1 dose previously as compared with 16% in the IVIG group.

The current study has limitations, most notably we used an in vitro exploratory study of patient plasma samples and their activity against superantigens produced by selected clinical strains to evaluate the effect of IVIG dose administered. Also, the low number of placebo-treated patients with GAS infection from the INSTINCT trial, and hence limited number of plasma samples for comparison, was a limitation. To enable comparison, samples from nonrandomized IVIG- and non–IVIG-treated patients within the INFECT cohort were included. A strength of this material is that all patient enrollment and sampling were

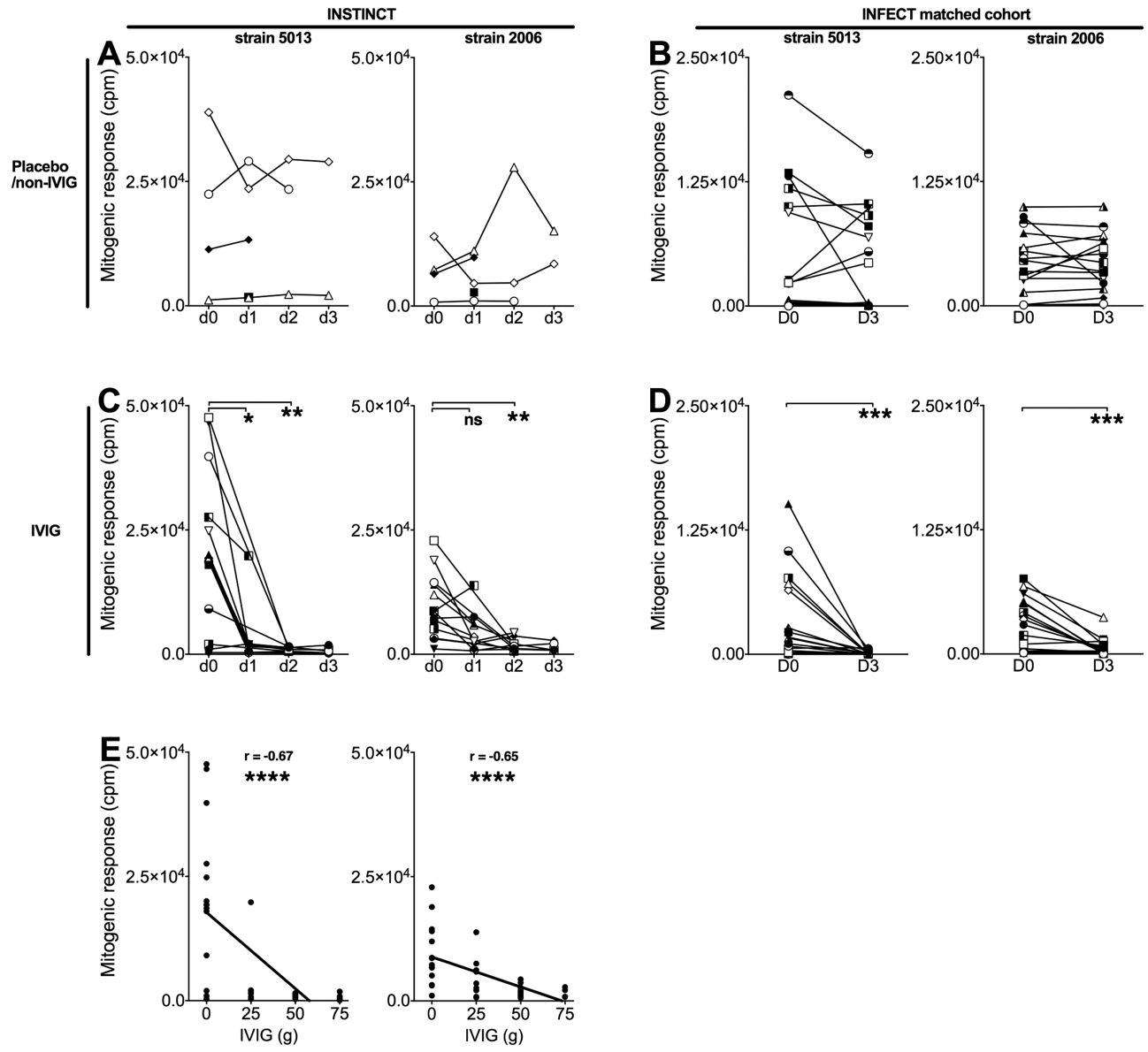

Figure 1. Plasma neutralizing activity towards superantigenic activity of GAS NSTI strains. Proliferative responses (cpm) of PBMCs to culture supernatants of GAS strains 2006 and 5013 were determined in the presence of plasma from INSTINCT patients randomized to administration of placebo (n = 5) (*A*) or IVIG (n = 14) (*C*) over 3 days. For comparison, plasma from non–IVIG-treated (n = 16) (*B*) or IVIG-treated (N = 16) (*D*) patients from the INFECT cohort were tested. Note the overlap between dose 1 responses of 2 patients (△ and ■) in panel *A*. The patient indicated by "■" had only 1 sample collected. Note that the *y* axes in panels *A* and *C* differ from those in *B* and *D* due to different PBMC donor responses. Wilcoxon matched-pairs signed rank test determined statistical significance in differences observed between the groups. *E*, Plasma neutralization in relation to dose of IVIG administered was possible in the INSTINCT patient cohort due to the precise sampling on each treatment day. Correlation was assessed through nonparametric Spearman's rank correlation coefficient. For INSTINCT patients: d = dose; for INFECT patients: D = day of sampling. D/d 0 = enrollment. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$); ns denotes $P = 0.08$. All results were reproduced using PBMCs from an additional donor (data not shown). Abbreviations: cpm, counts per minute; GAS, group A streptococcal; IVIG, intravenous immunoglobulin; ns, not significant; NSTI, necrotizing soft tissue infection; PBMC, peripheral blood mononuclear cell.

conducted using identical inclusion criteria and standard operating procedures for sampling.

The benefit of IVIG in GAS NSTIs remains to be demonstrated. A randomized trial targeting this patient subgroup is challenging as these infections are relatively rare and rapidly progressing and early diagnosis is often difficult. The INSTINCT trial and this study support such a trial and provide important information regarding the design, patient selection, and IVIG dosage. This study provides, for the first time, data on IVIG dosage in relation to mechanistic efficacy. Dosage studies of IVIG for acute infections are lacking; consequently, varying dosages have been used in different studies. In the trial of Darenberg et al [8], 1 g/kg bodyweight was used on day 1, followed by 0.5 g/kg on days 2 and 3. The observational study of Linnér et al [10] reported 0.5 g/kg bodyweight for 1–3 days in most patients, whereas Kaul et al [9] reported up to 2 g/kg.

Here we show data on mechanistic efficacy in relation to dose, demonstrating that 25 g may provide a high degree of plasma superantigen neutralization. Notably, analyses of patients with GAS NSTIs in the observational INFECT study showed an association between patients who had not received IVIG and 90-day mortality [12]. Based on these findings, we find it reasonable to propose the following dosage regimen: 0.5 g/kg bodyweight (or a minimum of 25 g) on day 1, followed by fixed doses of 25 g daily for 1 to 2 additional days. The higher dose on day 1 is warranted by the fact that 25 g was only borderline protective towards some strains in combination with often high levels of toxins and bacterial load at this time point and at the tissue site. This treatment regimen including number of treatment days should be evaluated in a future clinical trial.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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