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Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkl437
Advance Access publication 24 October 2006

Comment on: Quinolone resistance determinant qnrA3 in clinical isolates of Salmonella in 2000–2005 in Hong Kong
Matthew J. Ellington1* and Neil Woodford2

1Staphylococcal Reference Unit, Laboratory of Health-Care Associated Infection, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ, UK; 2Antibiotic Resistance Monitoring and Reference Laboratory, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ, UK

Keywords: plasmidic resistance, toxin–antitoxin, plasmid addiction

*Corresponding author. Tel: +44-20-8327-7259; Fax: +44-20-8200-7449; E-mail: matthew.ellington@hpa.org.uk

Sir,

In their recent correspondence, Chu et al.,1 noted the first observation of qnrA3 in salmonelae in Hong Kong,3 underlining the spread and diversity of plasmidic quinolone-resistance determinants worldwide. Such determinants now include qnrA1–5, qnrB and qnrS, some of which, at least, protect DNA gyrase,2 as well as the fluoroquinolone-acetylating variant of the aminoglycoside-modifying enzyme AAC(6′)Ib-cr.3 These proteins may influence the development of high-level resistance to at least some fluoroquinolones. In our recent article,5 we sought to explore the unexplained significant association between chromosomal fluoroquinolone resistance and plasmidic resistances to other antibiotic classes (such as ESBLs), which is seen in several bacterial genera.

We were pleased to note that Chu et al.1 cited our article,4 but would like to clarify a misinterpretation of our hypothesis. Multiresistance plasmids can encode toxin–antitoxin systems to prevent their own loss from the cell, and some toxins target DNA gyrase. We asked whether the development of quinolone resistance by plasmid-bearing strains might be associated with such DNA gyrase-targeting toxins. We speculated on whether a cell might escape addiction to toxin–antitoxin systems by developing resistance to the toxin. Mutational events in DNA gyrase could play a role in cells escaping addiction to plasmids with gyrase-targeting toxins, and we suggested that these mutations might fortuitously confer a degree of cross-resistance to quinolones. Notably, whilst at least three qnrA variants likely originated from the chromosome of Shewanella algaes,5 and although Qnr-type proteins inhibit/protect DNA gyrase,6,7 their precise cellular role(s) remains unknown. We would like to emphasize that we did not state, or wish to imply, that Qnr proteins could be part of a toxin–antitoxin pair as was attributed to our article by Chu et al. Rather, we postulated that gyrase ‘inhibitors/protectors’ such as Qnr might give some cross-resistance to gyrase-targeting toxins in addition to the more obvious resistance to quinolones. By so doing these proteins might open a ‘window’ and influence the frequency at which cells could eject the toxin–antitoxin encoding replicons without suffering apoptosis, thereby providing the host organism with a toxin–antitoxin evasion mechanism. This might provide a competitive advantage in demanding psychrophilic aquatic environments, the natural environment of S. algaes, by allowing enhanced ejection of ‘accessory’ replicons.

Transparency declarations

None to declare.

References


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Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkl430
Advance Access publication 28 October 2006

Comment on: Human intravenous immunoglobulin for experimental streptococcal toxic shock: bacterial clearance and modulation of inflammation
Govindarajan Rajagopalan1*, Robin Patel2, Srinivasa V. Kaveri3 and Chella S. David1

1Department of Immunology, Mayo Clinic College of Medicine, 200 First Street, SW, Rochester, MN 55905, USA; 2Divisions of Infectious Diseases and Clinical Microbiology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA; 3INSERM Unité 681 and Université Pierre et Marie Curie, Institut des Cordeliers, Paris, France

Keywords: superantigens, IVIg, HLA class II transgenic mice

*Corresponding author. Tel: +1-507-284-8180; Fax: +1-507-266-0981; E-mail: rajagopalan.govindarajan@mayo.edu
Sir,

In a recent report, Sriskandan et al.,\textsuperscript{1} evaluated human intravenous immunoglobulin (IVIg) in streptococcal toxic shock using the HLA-DQ8 transgenic mouse model. They observed that human IVIg neutralized streptococcal superantigens \textit{in vitro} as well as \textit{in vivo} and concluded that human IVIg could have potential therapeutic benefit in streptococcal toxic shock syndrome.\textsuperscript{1} Using a similar system we have observed that IVIg did not neutralize purified streptococcal superantigen-induced lymphocyte proliferation \textit{in vitro} as well as \textit{in vivo}. While Sriskandan et al.,\textsuperscript{1} used streptococcal bacterial culture supernatants as the source of bacterial superantigens, we used purified individual streptococcal bacterial superantigens for our study.

For \textit{in vitro} studies, splenic mononuclear cells, collected from naive A/Jo, HLA-DQ8 transgenic mice,\textsuperscript{2} were cultured with medium alone, indicated concentrations of superantigens or superantigen plus IVIg (1 mg/mL) for 48 h. Cell proliferation was determined by a standard thymidine incorporation assay. IL-2 and IFN-γ present in the culture supernatant were quantified by sandwich ELISA. The following purified superantigens were used \textit{in vitro} (Toxin Technology, Sarasota, FL, USA): streptococcal pyrogenic exotoxin (SPE) A, SPEB (not a superantigen, used as an internal control), SPEC, streptococcal mitogenic exotoxin (SME) Z2 (a gift from John D. Fraser, University of Auckland, New Zealand) and staphylococcal enterotoxin B (SEB). As sources of IVIg, we used several commercially available IVIg preparations including Tegeline (LFB, France), Octagam (Octapharma, Austria), Immunovenin (Bulgaria), Gammmagard (Baxter) and Endobulin (Immuno, Austria). All experiments were approved by the Mayo Clinic Institutional Animal Care and Use Committee.

As shown in Figure 1(a), IVIg, even at a 1000- to 10,000-fold higher concentration than the superantigens, was not capable of abolishing superantigen-induced T cell proliferation and nor was it capable of suppressing superantigen-induced IL-2 and IFN-γ production in the culture supernatants (not shown).

Next, we evaluated the ability of IVIg to neutralize superantigen activity \textit{in vivo}. HLA-DQ8 transgenic mice were challenged with 10 µg of SPEA or SMEZ\textsubscript{2} intraperitoneally followed by 1 g/kg of IVIg, also given intraperitoneally. PBS-challenged mice and experimental mice were sacrificed on day 3 and the distribution of CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells expressing specific TCR V\textbeta families was analysed by flow cytometry using commercial antibodies. As shown in Figure 1(b) (SPEA) and Figure 1(c) (SMEZ), administration of IVIg did not reduce the extent of superantigen-induced expansion of either CD4\textsuperscript{+} or CD8\textsuperscript{+} T cell subsets expressing specific TCR V\textbeta families. SPEA predominantly stimulates T cells expressing TCR V\textbeta8 but not TCR V\textbeta6 or 11. Accordingly, HLA-DQ8 mice challenged with SPEA showed a significant increase in the TCR V\textbeta8\textsuperscript{+} T cells in both CD4\textsuperscript{+} and CD8\textsuperscript{+} subsets. HLA-DQ8 transgenic mice challenged with SPEA and administered IVIg showed similar or even slightly higher expansion of TCR V\textbeta8 T cells in both CD4\textsuperscript{+} and CD8\textsuperscript{+} subsets, indicating that IVIg did not neutralize immune activation by SPEA. All IVIg preparations lacked suppressive activity against SPEA \textit{in vivo}. The same was true for SMEZ\textsubscript{2} which primarily activates T cells expressing TCR V\textbeta11.

We have shown in previous studies that while systemic administration of bacterial superantigens causes expansion of peripheral mature T cells, it causes massive apoptosis of CD4\textsuperscript{+}CD8\textsuperscript{+} double positive thymocytes in the thymus. We observed that administration of IVIg did not rescue superantigen-induced CD4\textsuperscript{+}CD8\textsuperscript{+} double positive thymocyte apoptosis \textit{in vivo} in HLA-DQ8 transgenic mice (data not shown). We have shown previously that HLA-DQ8 transgenic mice with targeted disruption of the IL-10 gene are extremely sensitive to bacterial superantigen-induced thymocyte apoptosis \textit{in vivo}. We administered IVIg in streptococcal toxic shock syndrome mice challenged with 10\textsuperscript{8} SPEA or SMEZ\textsubscript{2} intraperitoneally, followed by 1 g/kg of IVIg, also given intraperitoneally. Mice were sacrificed 3 days later and the distribution of different T cell subsets was analysed by flow cytometry. Each bar represents data from a minimum of four mice.

\textbf{Figure 1.} Effect of IVIg on superantigen-induced T cell activation \textit{in vitro} and \textit{in vivo}. (a) Single-cell suspensions of splenocytes from HLA-DQ8 transgenic mice were cultured for 48 h in the presence of medium alone or indicated superantigens. IVIg was added at a concentration of 1 mg/mL. Splenocyte proliferation was determined by measuring tritiated-thymidine uptake. HLA-DQ8 transgenic mice were challenged intraperitoneally with 10 µg of SPEA (b) or SMEZ (c), immediately followed by intraperitoneal injection of 1 g/kg of IVIg. Mice were sacrificed 3 days later and the distribution of different T cell subsets was analysed by flow cytometry. Each bar represents data from a minimum of four mice.

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\textbf{Figure 1.} Effect of IVIg on superantigen-induced T cell activation \textit{in vitro} and \textit{in vivo}. (a) Single-cell suspensions of splenocytes from HLA-DQ8 transgenic mice were cultured for 48 h in the presence of medium alone or indicated superantigens. IVIg was added at a concentration of 1 mg/mL. Splenocyte proliferation was determined by measuring tritiated-thymidine uptake. HLA-DQ8 transgenic mice were challenged intraperitoneally with 10 µg of SPEA (b) or SMEZ (c), immediately followed by intraperitoneal injection of 1 g/kg of IVIg. Mice were sacrificed 3 days later and the distribution of different T cell subsets was analysed by flow cytometry. Each bar represents data from a minimum of four mice.
superantigen-induced toxic shock and mortality.\textsuperscript{3} Mortality in DQ8.II-10/– mouse challenged with superantigen alone was no different from the group challenged with superantigen along with IVIg treatment (SPEA alone, 2/2; SPEA+IVIg, 3/4; SEB alone, 4/4; SEB+IVIg, 3/3). We determined whether administration of IVIg prior to superantigen challenge would have any protective effect on superantigen-induced immune activation and mortality. For this, we first administered 1 g/kg of IVIg intraperitoneally in to HLA-DQ8 and HLA-DQ8.II-10/– mice, followed 30 min later by 10 \(\mu\)g of SPEA. The extent of T cell expansion was comparable between HLA-DQ8 transgenic pretreated with IVIg or not (data not shown). Similarly, the extent of mortality was similar in HLA-DQ8.II-10/– mice either pre-treated or not with IVIg (3/3 in each group).

We also screened the ability of IVIg to neutralize the staphylococcal superantigen, SEB using HLA-DR3 as well as HLA-DQ8 transgenic mice. IVIg did not show any significant neutralization of SEB activity \textit{in vitro} (Figure 1a) or \textit{in vivo} (data not shown), similar to our previous findings.\textsuperscript{4} Sriskandan \textit{et al}.,\textsuperscript{5} also showed that IVIg preparations can have opsonizing activity which may help clear bacterial infection. However, our previous study indicated a lack of benefit of IVIg in a murine model of group A streptococcal necrotizing fasciitis.\textsuperscript{5}

Bacterial superantigens generally have two modes of binding to MHC class II molecules; low affinity or generic binding and high affinity or zinc-dependent binding.\textsuperscript{6} To be effective, the natural antibodies present in IVIg should be of sufficiently high affinity to prevent high affinity interactions between extremely small amounts of bacterial superantigens and their abundantly present ligands, MHC class II molecules.\textsuperscript{6} Results of our studies differ from those of Sriskandan \textit{et al}.,\textsuperscript{4} The IVIg preparations we studied were different from the preparation studied by Sriskandan \textit{et al}.,\textsuperscript{1} (Endobulin S/D, Baxter) and it has been recently shown that the ability to neutralize various bacterial superantigens varies among different IVIg preparations.\textsuperscript{7} Alternatively, differences may relate to the use of purified superantigens versus bacterial culture supernatants.

Acknowledgements

We thank Julie Hanson and her crew for excellent mice husbandry. This study was supported by NIH grant AI14764.

Transparency declarations

GR, RP and CSD have none to declare. SVK has received grants in the last 3 years to study various aspects of the mechanisms of autoimmune and inflammatory diseases from LFB, ZLB Behring and Talecris Biotherapeutics.

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Correspondence

Shirane Sriskandan*

Department of Infectious Diseases and Immunity, Hammersmith Hospital, Imperial College London, Du Cane Road, London W12 0NN, UK

Keywords: bacterial infections, models, septic shock, \textit{Streptococcus pyogenes}, immunology, immunomodulators

*Corresponding author. Tel: +44-208-383-3135; Fax: +44-208-383-3394; E-mail: s.sriskandan@imperial.ac.uk

Sir,

The failure of Rajagopalan \textit{et al}.,\textsuperscript{1} to demonstrate any effect of intravenous immunoglobulin (IVIg) \textit{in vitro} is perplexing, as several investigators have demonstrated the inhibitory properties of IVIg on superantigen-induced T cell mitogenesis and blast formation \textit{in vitro}.\textsuperscript{2–6} Although variation in IVIg source may partly explain their results, the correspondents used only a single concentration of IVIg (1 mg/mL) in their \textit{in vitro} tests and therefore cannot draw firm conclusions about activity. Furthermore, they used higher concentrations of superantigen than were present in \textit{Streptococcus pyogenes} superantigen used in our study.\textsuperscript{7}

Using the same batch of IVIg used in our original report,\textsuperscript{7} IVIg 0.5 mg/mL was sufficient to cause 50% inhibition of 10 ng/mL staphylococcal enterotoxin B (SEB)- or streptococcal pyrogenic exotoxin A (SPEA)-induced T-cell proliferation. Importantly, when the concentration of SEB was raised to 10 \(\mu\)g/mL, inhibition required concentrations of 5 mg/mL IVIg (L. Faulkner and S. Sriskandan, unpublished data), illustrating the importance of