Immunology Letters 143 (2012) 146–151

Contents lists available at SciVerse ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Intravenous immunoglobulin treatment on anti-GM1 antibodies associated neuropathies inhibits cholera toxin and galectin-1 binding to ganglioside GM1

Wei Peng[a,b,*]

[a] Shanghai XuHui Centre Hospital, 966 HuaiHai Zhong Road, Shanghai 200031, PR China
[b] Laboratoire d'Immunologie, Faculté de Médecine de Marseille, Université de la Méditerannée, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France

ARTICLE INFO

Article history:
Received 6 November 2011
Received in revised form
30 December 2011
Accepted 13 January 2012
Available online 23 January 2012

Keywords:
Anti-GM1 antibodies
Intravenous immunoglobulin
Galectin-1
Cholera toxin

ABSTRACT

Intravenous immunoglobulin (IVIg) therapy is efficacious in some peripheral nervous autoimmune diseases associated with anti-GM1 antibodies. Numerous mechanisms of action have been proposed to account for the immunomodulatory effects of IVIg in immune-mediated diseases. Up to now, the mechanisms of action of IVIg in pathology associated with anti-GM1 antibodies have not been well documented. In the present study, we discovered that IVIg did not inhibit the binding of anti-GM1 antibodies to its antigen and IVIg perfusions did not reduce anti-GM1 antibodies titers. In this observation, we have the result different from the hypothesis of presence of anti-idiotypic antibodies in different IVIg preparations, and show that IVIg inhibits the binding of cholera toxin and galectin-1 to GM1-expressing cells using flow cytometry. Our results suggest that the correct ratio galactosyl/agalactosyl IgG in IVIg interact with macrophages receptors to down-regulate inflammatory function of macrophages and autoimmune diseases in peripheral nerve system.

1. Introduction

Intravenous immunoglobulin (IVIg) therapy is efficacious in some nervous autoimmune diseases including multifocal motor neuropathy (MMN), lower motor neuropathy without conduction block (LMN), acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor axonal neuropathy (AMAN), chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) and multiple sclerosis (MS) [1–5]. IVIg, prepared from pooled normal polyspecific IgG, may act on the immune-mediated disease by multiple mechanisms depending on their Fab or Fc part such as anti-idiotypic antibody effect or Fc receptor blockade [6,7].

MMN, LMN and AMAN are frequently associated with antibodies anti-GM1 IgM, IgG or IgA. Frequency of these antibodies varied among laboratories according to the detective methods [8]. GM1 is present in motor neurons, dorsal root ganglion cells and sensory axons [9]. It is mainly localized to and around the nodes of Ranvier near the sodium and potassium channels [10]. At present, pathogenicity of anti-GM1 antibodies is still controversial and the mechanisms of peripheral nerve system injury are still unknown. Anti-GM1 antibodies may directly cause disease by binding to GM1 or cross-reactive neuronal antigens with consequent activation of proinflammatory pathways or by creating a functional block of GM1-dependent processes: for instance, anti-GM1 antibodies could directly alter the ion channel functions [11]. Some claims that the attack by anti-GM1 antibodies requires nodal structure abnormalities [12,13]. β-Subunit of cholera toxin is known to specifically and efficiently bind to GM1. Different endogenous GM1-ligands have been proposed: (i) using anti-idiotypic antibodies, Riggott et al. have identified four GM1 binding proteins in mammalian bilayer [14]; (ii) VIP21 (caveolin-1), an essential structure of caveolin [15]; and (iii) galectin-1, a β-galactoside-binding protein reported as a major ligand of GM1 [16]. The last one is over-expressed during inflammatory processes on activated macrophages [17] and tumor growth [18], besides galectins have immunomodulatory and growth regulatory activities [19,20].

Mechanisms of action of IVIg in pathology associated with anti-GM1 antibodies have not been well documented. Blockage of antibodies by anti-idiotype antibodies presenting in IVIg has been proposed as an action mechanism [21,22], and more recently, it is hypothesized that IVIg may reduce anti-GM1 antibody-mediated complement deposition [23,24], however these mechanisms were not convincingly demonstrated. Moreover, Yuki and Miyagi, using ELISA described that IVIg interacted with peptide epitope of cholera toxin and inhibited the binding of cholera toxin to GM1 in this manner [25].

To explore the mechanisms of action of IVIg during anti-GM1 antibodies associated diseases, we tested two hypotheses: (i) presence of anti-idiotypic antibodies in different IVIg preparations; (ii) inhibition by IVIg of cholera toxin and galectin-1 binding to GM1-expressing cells using flow cytometry.

* Correspondence address: Shanghai XuHui Centre Hospital, 966 HuaiHai Zhong Road, Shanghai 200031, PR China.
E-mail address: pengwei39@hotmail.com

0165-2478/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2012.01.005

2. Materials and methods

2.1. Reagents and cell lines

Three different products of human IVIg (50 mg/ml): Sandoglobuline® (Novartis, Rueil Malmaison, France), Tegeline® (LFB, Paris, France) and Endoglobuline® (Baxter, Maurepas, France) were used. $F(ab')_2$ IVIg preparation was provided by Srini V. Kaveri (INSERM U430, Paris, France). Purified monosialoganglioside GM1 from bovine brain was purchased from Sigma (St Quentin Fallavier, France) and dissolved in methanol. The high affinity GM1-specific-ligand biotinylated cholera toxin β-subunit (0.5 mg/l) was purchased from Sigma (St Quentin Fallavier, France). Human biotinylated galectin-1 (0.5 mg/ml) was the gift of HJ Gabius (Munich, Germany). FITC-conjugated $F(ab')_2$ goat anti-human IgG and anti-human IgM (1 mg/ml) were purchased from Biorad (Ivry sur Seine, France), horseradish peroxidase-linked goat anti-human IgM (Fcμ) and anti-human IgG (Fc) (1 mg/ml) from Beckman-Coulter (Paris, France), streptavidine–allophycocyanin (Sav–APC) (0.2 mg/ml) from Becton Dickinson (Pont-de Claix, France) and steptavidine–fluorescein-isothiocyanate (Sav–FITC) (1 mg/ml) from Dako (Trappes, France).

The human neuroblastoma cell line SKN-MC, which significantly express endogenous GM1, and the human glioblastoma cell line U229 treated or not with exogenous GM1 were obtained from the American Type Culture Collection (Manassas, VA). These cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 UI/ml penicillin and 100 μg/ml streptomycin. Cells were harvested in presence of 0.05% trypsin–0.02% EDTA (Invitrogen, Cergy Pontoise, France).

2.2. Patients' sera

Diagnoses assessed by the clinical and neurophysiological examinations were performed in two neurological departments in Marseilles. Most of the patients have been previously reported [1,26,27]. All patients gave written informed consent, and the local ethics committee approved the study. All sera were aliquoted, frozen and stored at −80 °C until used. Sera were subdivided in four groups: MMN ($n = 13$), AMAN ($n = 2$), LMN ($n = 4$) and cryptogenic partial epilepsies (CPE) ($n = 4$) associated with anti-GM1 antibodies. The last group's sera have been selected among epileptic patients for the presence of either IgG or IgM anti-GM1 antibodies [27]. Patient's groups and antibodies testing were summarized in Table 1.

2.3. Enzyme-linked immunosorbent assay (ELISA)

ELISA was conducted according to a modified procedure described by Adams et al. [28]. Each well of the non-irradiated microtiter plates (Sayag, Courtaboeuf, France) was filled with 0.5 μg of GM1 diluted in methanol or without GM1. After complete evaporation at 37 °C, they were saturated for 30 min at 4 °C with washing buffer (PBS pH 7.2 containing 0.05% Tween 20 (Sigma, St Quentin Fallavier, France). All the incubations were performed in duplicate with the washing buffer. Both of the diluted negative and positive control samples together with the diluted patient samples (1/20) were then incubated overnight at 4 °C. After five washes, horseradish peroxidase-linked goat anti-human IgM (1/25,000) or IgG (1/20,000) were added and incubated for 2 h at room temperature. Plates were washed and developing solution (0.1 M sodium citrate, 0.1 M citric acid, 1 mg/ml ortho-phenylenediamine and 3% H_2O_2) was added. After 30 min, reaction was stopped with H_2SO_4 4 M. Non GM1 coated wells were used as blank controls. Optical densities (OD) were normalized with OD obtained on blank wells at 490 nm. Means of the results obtained with controls plus two standard deviations gave the positive thresholds. All sera giving a value above the thresholds were serially diluted. ELISA results were expressed by the inverse of the last dilution given a value above the thresholds.

For competitive experiments, sera were diluted at 1/40 or 1/80 in PBS Tween 0.05% and incubated with IVIg or myeloma IgG at concentration ranging from 0.1 mg/ml to 25 mg/ml. After 3 h incubation at 37 °C, the presence of anti-GM1 antibodies was detected using ELISA in the same condition described before.

2.4. Flow cytometry analysis

SK-N-MC and U229 treated or not with exogenous GM1 were incubated for 15 min at 4 °C either with diluted normal human serum (1/20) working as negative control, or with patients' sera diluted at 1/20, biotinylated cholera toxin β-subunit (0.5 μg/ml) or biotinylated galectin-1 (20 μg/ml) in 50 μl PBS, BSA 0.01%, sodium azide 0,02% alone or in addition with IVIg or $F(ab')_2$. After wash, another 15 min incubative step was started to reveal anti-GM1 patients' sera, cholera toxin or galectin-1 binding respectively in presence of either 10 μg/ml FITC-labelled anti-IgM, anti-IgG, or streptavidin-FITC or streptavidin–APC in 50 μl of PBS, BSA 0.01%, and sodium azide 0.02%. After two washes, cells were fixed in PBS 2% paraformaldehyde. Fluorescence analysis was conducted by a FACS Calibur cell analyzer (Becton Dickinson, Mountain View, CA).

3. Results

3.1. IVIg did not inhibit binding of anti-GM1 antibodies to its antigen

We did not detect anti-GM1 IgG reactivity in the three IVIg preparations. Moreover, adding IVIg did not increase non-specific ELISA signal. Competitive ELISA was then performed in presence of different concentrations of Sandoglobulin® (0.1 mg/ml to 25 mg/ml) with all sera of patients suffering from MMN, LMN, AMAN or CPE. IVIg never reduced anti-GM1 IgG or IgM reactivity. This observation was confirmed through using IVIg from other companies (Tegeline® or Endoglobuline®) with 6 sera samples of patients suffering from MMN, LMN or CPE. Moreover, different ELISA experimental conditions were designed for two representative patients suffering from MMN: serial sera dilutions during pre-incubation step, temperature and time of IVIg incubation, presence or absence of detergent. In none of these experiments, were we

Table 1
Immunological characteristics of patients used in this study.

Diagnosis	IgM anti-GM1		IgG anti-GM1	
	Number of patients	Range of titers	Number of patients	Range of titers
MMN ($n = 13$)	13	80–640	2	80
LMN ($n = 4$)	3	320–25,600	3	160–1280
AMAN ($n = 2$)	0	None	2	640–1280
CPE ($n = 4$)	3	160–320	2	80

MMN, multifocal motor neuropathy; LMN, lower motor neuropathy without conduction block; AMAN, acute motor axonal neuropathy; CPE, cryptogenic partial epilepsies.