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Overview of IgG-Reactivity in Therapeutic immunoglobulins Revealed by Protein Array Analysis

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Abstract

In addition to therapy for immune deficient patients, intravenous immune globulins (IVIg) are used to treat several autoimmune and inflammatory diseases. Several groups working to better understand how IVIg can modulate immune and inflammatory responses have proposed the involvement of self-reactive IgG present in IVIg. Consequently, a more exhaustive characterization of IVIg repertoire could be very helpful for their better utilization. To increase our knowledge on IVIg repertoire, we have used the technology of protein array analysis to characterize a commercial IVIg preparation by screening its interaction with 9484 proteins of human origin. As a result, we have identified 67 proteins, which are strongly recognized by the IgG present in IVIg. Most of these targets are highlighted for the first time bringing new insight into IVIg mechanisms of action.

Keywords: Immunomodulation; Therapeutic immunoglobulins; IgG self-repertoire; Human proteins

Abbreviations: LIM: named after their initial discovery in the proteins Lin11 Isl-1 Mec-3; PDZ: postsynaptic density protein-95/disks large/zonula occludens-1; PDLIM2: PDZ-LIM domain-containing protein 2.

Introduction

Intravenous immunoglobulins (IVIg) represent pooled IgG of thousand blood donors; IVIg are replacement immunoglobulins for immunodeficient patients and have positive modulatory effects in autoimmune and inflammatory disorders (reviewed in [1,2]). Their IgG repertoire is polyclonal, representative of both adaptive and natural immunity and contains self-reactive antibodies [3-5]. IVIg contain antibodies directed against several molecules, for example CD4, CD8, HLA, cytokines, T and B cell receptors, heat-shock proteins (reviewed in [6]). Its mechanisms of action have been extensively investigated. IVIg can proceed through direct interaction with immune cells or indirectly by binding to soluble messengers. It can act upon BCR, TCR and Fc receptors activation [1,5] and can also be related to down-modulation of CD16 (FcγRIII) on macrophages in Kawasaki disease [2,7].

Until recently, information about IVIg self-reactivity came from studies focusing on particular diseases. Recently, an extensive study based on mass spectrometry analysis have highlighted that IVIg can bind to several intracellular proteins [3]. This group have done proteomic analysis of IVIg interaction on protein extracts prepared from freshly isolated human umbilical vein endothelial cells and from a cell line derived from human laryngeal carcinoma [3]. A total of 96 intracellular proteins, revealed as specific targets for IVIg, were related to various functions such as chaperones, cytoskeletal proteins, DNA, RNA and protein processing, oxydo-reduction, glycolysis as well as growth regulation. Moreover, fourteen of these IVIg-target proteins were underscored in both cell extracts. In line with the current efforts to further characterize IVIg self-reactivity, we have thus performed antibody-profiling based on protein microarray technology.

Materials and Methods

Human protein microarray

Autoreactivity of a commercial intravenous immunoglobulin preparation (IVIg) (GamunexTM, Talecris Biotherapeutics ltd., Toronto, ON, Canada) was determined by Antibody specificity profiling service of Invitrogen (Carlsbad, USA). IVIg were probed by Invitrogen at 0.1 µg/ml and 1.0 µg/ml. A total number of 9484 human proteins were screened using the ProtoArray Human Protein Microarrays v5.0. Data analysis was done by Invitrogen using ProtoArray Prospector software. Average background fluorescence values were respectively 107 and 112 (RFU; relative fluorescence unit). The three criteria, according to Invitrogen profiling service experimental design, permit to determine the more significant interaction between IVIg and the different proteins. Briefly, the negative control RFU for each protein was determined by incubation with probing buffer in absence of IVIg and detection by the goat anti-human IgG. Firstly, for each of the 9484 proteins, the IVIg signal value had to be greater than 2000 RFU and more than 2-fold higher than the negative signal. Secondly, the second negative control signal value had to be less than 1000 RFU and thirdly the replicate spot coefficient of variation (CV) had to be less than 50%.

Results and Discussion

Among the 9484 proteins tested, IVIg have a significant interaction with 67 peptides from 66 proteins based on the three criteria described above (Table 1). As previously reported by Bussone et al. [3], most of the interactors were of intracellular origin, except for CD16a, whose two independent peptides were recognized by IVIg. Among these interactor-proteins, we also spotted PDZ-LIM domain-containing protein 2 (PDLIM2) and LIM domain protein 1, which was one of the 96 proteins identified by Bussone's group, but was not included in their final list of 14 targets. Interaction of IVIg with CD16a

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Database ID	IVIg (RFU)	CV (%)	Neg (RFU	Fold	Description
BC017865.1	10582	3	267	39,6	Fc fragment of IgG, low affinity IIIa, receptor (CD16a) (FCGR3A) (*)
NM_024826.1	7057	4	289	24,5	Microtubule-associated protein 9 (*)
BC064612.1	3540	1	254	14,0	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 6 (MLLT6) (*)
NM_023930.1	5321	3	409	13,0	Potassium channel tetramerisation domain containing 14 (KCTD14)
NM_004987.3	3450	1	267	12,9	LIM and senescent cell antigen-like-containing domain protein 1
BC001553.1	2386	3	241	9,9	Chromatin modifying protein 2B (CHMP2B)
BC022893.1	6805	7	737	9,2	Potassium channel tetramerisation domain containing 6 (KCTD6)
BC036723.1	2990	1	348	8,6	Fc fragment of IgG, low affinity IIIa, receptor (CD16a) (FCGR3A)
BC000393.1	7147	1	838	8,5	Family with sequence similarity 127, member B (FAM127B)
NM_018184.1	3304	18	403	8,2	ADP-ribosylation factor-like 8B (ARL8B) (*)
NM_201403.1	4039	9	557	7,3	MOB1, Mps One Binder kinase activator-like 2C (yeast) (MOBKL2C),
BC017202.2	3812	0	549	6,9	Isovaleryl-CoA dehydrogenase, mitochondrial (*)
NM_021630.4	4018	0	607	6,6	PDZ and LIM domain protein 2
NM_201613.1	4095	6	626	6,5	IKK interacting protein (IKIP), transcript variant 3.1
BC022893.1	6019	10	936	6,4	Potassium channel tetramerisation domain containing 6 (KCTD6)
BC030253.1	2376	4	387	6,1	Glutathione S-transferase M3 (brain) (GSTM3)
BC007346.2	5822	6	955	6,1	Uncharacterized protein C16orf14
BC048099.1	2197	7	364	6,0	Zinc finger and SCAN domain containing 1 (ZSCAN1) (*)
NM_003302.1	2428	4	411	5,9	Thyroid hormone receptor interactor 6 (TRIP6)
NM_005440.3	4311	2	752	5,7	Rho family GTPase 2 (RND2)
NM_001004286.1	5142	2	913	5,6	DNA fragmentation factor subunit beta
NM_012094.3	2445	1	443	5,5	Peroxiredoxin 5 (PRDX5), nuclear gene encoding mitochondrial protein,
NM_012395.2	2179	12	404	5,4	PFTAIRE protein kinase 1 (PFTK1)
NM_003130.1	2072	2	384	5,4	Sorcin (SRI), transcript variant 1
NM_207350.1	2704	16	520	5,2	Similar to FRG1 protein (FSHD region gene 1 protein) (MGC72104)
BC003065.1	3476	9	672	5,2	Cell division protein kinase 2 (*)
BC033817.1	3722	8	738	5,0	PC4 and SFRS1 interacting protein 1 (PSIP1)
NM_014288.2	3104	3	621	5,0	Centromere protein R
NM_023940.1	3078	4	619	5,0	RAS-like, family 11, member B (RASL11B)
NM_004092.2	3823	2	772	5,0	Enoyl-CoA hydratase, mitochondrial
BC014385.1	2153	21	436	4,9	Integrin beta 3 binding protein (beta3-endonexin) (ITGB3BP)
NM_016848.2	2988	1	630	4,7	SHC-transforming protein 3
NM_197962.1	2813	2	618	4,6	Glutaredoxin 2 (GLRX2), transcript variant 2
NM_002927.3	2162	18	478	4,5	Regulator of G-protein signaling 13 (RGS13), transcript variant 1
BC009779.1	2537	2	563	4,5	Outer dense fiber of sperm tails 2-like (ODF2L)
NM_016816.1	3497	1	781	4,5	2',5'-oligoadenylate synthetase 1, 40/46kDa (OAS1), transcript variant 1
NM_005034.2	2009	3	478	4,2	Polymerase (RNA) II (DNA directed) polypeptide K, 7.0kDa (POLR2K)
NM_017451.1	3392	7	819	4,1	BAI1-associated protein 2 (BAIAP2), transcript variant 2
NM_012289.2	3919	17	994	3,9	Kelch-like ECH-associated protein 1
BC003549.1	3466	7	889	3,9	Enoyl Coenzyme A hydratase domain containing 1 (ECHDC1)
BC021189.2	2580	4	666	3,9	cDNA clone IMAGE:4829245
BC002914.1	2787	7	744	3,7	WAS/WASL-interacting protein family member 1
BC030592.2	2890	9	795	3,6	HIV-1 Rev binding protein (HRB)
BC009873.1	2144	11	592	3,6	cDNA clone IMAGE:3946787, partial cds
NM_023938.4	2013	5	566	3,6	Specifically androgen-regulated gene protein
NM_014583.2	3095	3	915	3,4	LIM and cysteine-rich domains 1 (LMCD1)
NM_203425.1	3139	0	930	3,4	Chromosome 17 open reading frame 82 (C17orf82)

Database ID	IVIg (RFU)	CV (%)	Neg (RFU)	Fold	Description
NM_002904.4	2140	11	637	3,4	RD RNA binding protein (RDBP)
BC030950.1	2343	1	721	3,3	Small nuclear protein PRAC
BC035582.1	2857	1	886	3,2	Tripartite motif-containing protein 22
BC009967.1	2091	8	682	3,1	N-terminal kinase-like protein
BC004259.1	2687	2	885	3,0	Chromosome 14 open reading frame 140 (C14orf140)
NM_016364.2	2719	8	897	3,0	Dual specificity phosphatase 13 (DUSP13), transcript variant 6
NM_144594.1	2135	3	726	2,9	Family with sequence similarity 112, member B (FAM112B)
NM_016096.1	2132	5	726	2,9	Zinc finger protein 706 (ZNF706), transcript variant 2
NM_144736.3	2688	1	919	2,9	Protein midA homolog, mitochondrial
NM_003161.1	2717	7	955	2,8	Ribosomal protein S6 kinase, 70kDa, polypeptide 1 (RPS6KB1)
BC009894.2	2465	3	869	2,8	3'-phosphoadenosine 5'-phosphosulfate synthase 2 (PAPSS2)
BC056911.1	2632	4	960	2,7	Dual specificity phosphatase 15 (DUSP15)
NM_015698.2	2036	1	747	2,7	G patch domain and KOW motifs (GPKOW)
BC034401.1	2132	0	787	2,7	cDNA clone IMAGE:5172086, partial cds
NM_005678.3	2516	6	973	2,6	SNRPN upstream reading frame (SNURF), transcript variant 1
PV3665	2237	1	892	2,5	Casein kinase 1, delta (CSNK1D), transcript variant 1
NM_006479.2	2029	7	821	2,5	RAD51 associated protein 1 (RAD51AP1)
NM_207356.1	2152	7	871	2,5	Chromosome 1 open reading frame 174 (C1orf174)
BC051000.1	2024	2	838	2,4	T-cell leukemia/lymphoma 1B (TCL1B)
BC033230.1	2189	5	943	2,3	Zinc finger protein 808 (ZNF808)

1. IVIg interactions were analyzed using 1.0 µg/mL. Data include IVIg signal (threshold >2000) corresponding to background subtracted pixel intensity value (RFU), the CV associated with the duplicate spots for each protein and the fold increase between IVIg signal versus negative signal (Neg) used for the corresponding protein in the negative control assay.
2. (*) Indicated significant interaction whith IVIg at 0.1µg/ml (using a Signal threshold greater than 1000 RFU).

Table 1: Therapeutic immunoglobulin pattern of interaction with human proteins.

(FcγRIIIa receptor) is the only one linked to an IVIg-related disease effect. Indeed, down-modulation of CD16a has been linked to IVIg treatment of patients with Kawasaki disease (KD) [8]. However, IVIg self-reactivity has been shown to target many other proteins present in the 9484 protein arrays, namely heat shock proteins, interleukin-1β, HLA-DR, CD64 and even influenza A virus antigen H3N2. In our opinion, the absence of reactivity for these well-known antigens appeared unlikely. IVIg repertoire have been estimated to recognize up to 107 antigenic epitopes [9], suggesting that less than 1000 antibody molecules might be available for each epitope when using IVIg at 1.0µg/mL. We then reconsidered the protein array data interpretation by using criteria to include as a positive internal control the reactivity against H3N2. We thus determine that he signal used for IVIg must be higher than 50 RFU, the negative signal must be higher than 1 RFU (to exclude negative values) and the ratio between those two signals must be higher than 5. Finally, the CV for duplicate must be less than 15%. Using these criteria we have observed that IVIg (1.0 µg/ml) was able to interact with 776 peptides, which included all 67 proteins listed in Table 1 (Supplemental data Table 1). Based on these criteria, IVIg were indeed found reactive for influenza A antigen (H3N2) [10,11], which was the only non-human antigen present in this array, showing 526 ± 7% RFU, which was 44-fold stronger than the negative signal. Similarly, we obtained acceptable reactivity for HLA-DR (59 ± 10% RFU; 39-fold), thyroglobulin (205 ± 10% RFU; 26-fold), CD64 ((755 ± 10%, 23-fold), interleukin-1β (251 ± 10%, 15-fold), lamin A/C (277 ± 14%, 8-fold) and ferritin (1177 ± 10%, 5-fold), which are all related to immunomodulatory properties of IVIg [2,3,7,8,12-14]. Down-modulation of CD16a and CD64 has been reported by many studies following IVIg therapy of patients suffering from KD and our results suggest that anti-CD16a and anti-CD64 antibodies could be involved in this mechanism. Overall, our observations bring at least 66 new candidates as strong IVIg interactions and also suggest that IVIg self-reactivity is still underestimated when considering the possibility of 776 protein targets underscored when using criteria based on previous observations. Finally, we hope that this information will be helpful for all groups working on IVIg by comparing their observations with our data, which might expose new routes for IVIg mechanisms of action.

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