Journal of Immunological Methods 425 (2015) 62–68

Contents lists available at ScienceDirect

Journal of Immunological Methods

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

Research paper

A rapid, accurate and robust particle-based assay for the simultaneous screening of plasma samples for the presence of five different anti-cytokine autoantibodies

Daniel Kring Rasmussen Guldager [a,1], Jakob Hjorth von Stemann [a,1], Rune Larsen [a], Jakob Thaning Bay [a], Pia Søndergaard Galle [a], Morten Svenson [b], Henrik Ullum [a], Morten Bagge Hansen [a,*]

[a] Department of Clinical Immunology and Blood Bank, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark
[b] Institute for Inflammation Research, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form 15 May 2015
Accepted 11 June 2015
Available online 14 June 2015

Keywords:
Autoantibodies
Multiplex assay
Blood transfusion
Antibody detection
Cytokine

ABSTRACT

Purpose: To establish and validate a rapid, cost-effective and accurate screening assay for the simultaneous testing of human naturally occurring anti-cytokine autoantibodies (c-aAb) targeting interleukin-1α (IL-1α), interleukin-6 (IL-6), interleukin-10 (IL-10), granulocyte–macrophage colony-stimulating factor (GM-CSF), and interferon α (IFNα). Because the c-aAbs can be transferred to patients through blood transfusion, the assay was used to assess c-aAb levels in a cohort of patients who were receiving blood transfusions and subsequently presented with or without febrile reactions.

Materials and methods: The microsphere-based Luminex platform was used. Recombinant forms of human IL-1α, IL-6, IL-10, GM-CSF, and IFNα were gently coupled to MAG-PLEX beads. Plasma IgG binding was measured with phycoerythrin (PE)-labeled secondary antibodies. Previously confirmed c-aAb positive and negative donor plasma samples and pooled normal immunoglobulin preparations were used to validate the assay. Plasma samples from 98 transfusion recipients, half of whom presented with febrile reactions, were tested by the assay.

Results: The assay detected specific and saturable immunoglobulin G (IgG) binding to each of the tested cytokines in previously confirmed c-aAb positive plasmas and in preparations of pooled normal immunoglobulin. Confirmed c-aAb negative plasmas gave no saturable binding. The detection limit of the cytokine autoantibodies was estimated to be between 1 pM and 10 pM. The recovery of confirmed cytokine autoantibodies quantities in the negative plasma samples ranged between 80% and 125%. The analytical intra- and inter-assay variations were 4% and 11%, respectively. Varying c-aAb levels were detectable in the transfusion recipients. There was no difference in c-aAb frequency between the patients with or without febrile transfusion reactions. The c-aAb level before and after the blood transfusions varied only slightly and in an irregular manner.

Conclusion: This assay simultaneously detected up to five different c-aAbs in pooled human IgG and in plasma from individual blood donors, and it was deemed suitable for larger screenings. Based on confirmed antibody binding characteristics and the resultant reactivity in this multiplex assay, a classification of the c-aAb levels was suggested. The screening results of the recipients who received blood transfusions indicate that more studies are needed to clarify the role of antibodies, if any, in transfusion medicine and in high-dose immunoglobulin treatment.

1. Introduction

High affinity and neutralizing c-aAbs have been reported in pharmaceutical preparations of pooled human IgG (Svenson et al., 1993). In accordance, substantial amounts of autoantibodies against IL-1α, IL-6, IL-10, GM-CSF, and IFNα have been reported in individual blood donor plasmas (de Lemos et al., 2009a). A clinical impact of c-aAbs has been suggested based on the association of alveolar proteinosis with c-aAbs against GM–CSF (Uchida et al., 2004), the association of c-aAbs against IL-6 with bacterial infections (Puel et al., 2008) and metabolic disturbances (Fosgerau et al., 2010) and an association of c-aAb against IL-1α with a less erosive development of rheumatoid arthritis (Svenson et al., 2000; de Lemos et al., 2009a).

It is well known that blood transfusion, in general, and high-dose immunoglobulin treatments, in particular, suppress or affect the immune system. This effect is likely to be multifactorial. A blood transfusion recipient often obtains multiple transfusions, and in theory, transfusion transmitted donor derived and neutralizing c-aAbs could

Abbreviations: IL, interleukin; IFN, interferon; GM–CSF, Granulocyte Macrophage–Colony Stimulating Factor; c-aAbs, cytokine autoantibodies; K_{av}, avidity constant; B_{max}, maximal antibody binding capacity; MFI, mean fluorescence activity.

* Corresponding author at: Department of Clinical Immunology and Blood Bank, Rigshospitalet, Copenhagen National University Hospital, Blegdamsvej 9, 2100 Ø, Copenhagen, Denmark.
E-mail address: bagge@dadlnet.dk (M.B. Hansen).

[1] These authors contributed equally to the publication.

http://dx.doi.org/10.1016/j.jim.2015.06.010
0022-1759/

accumulate in the recipient, thus causing an acquired cytokine deficiency, which eventually might compromise the immune system (Browne, 2014). The passive transfer of pathogenic antibodies during blood transfusion is a well-known phenomenon, e.g., donor allergen-specific IgE antibodies causing allergic reactions in the recipient, antibodies to the recipient's erythrocytes causing hemolysis, leukocyte antibodies causing transfusion-related acute lung injury, and platelet antibodies causing thrombocytopenia (Hansen et al., 2007). The question remains, however, concerning the extent to which c-aAbs are transferred by allogeneic blood transfusion and whether these passively transferred autoantibodies can have any pathophysiological role in the recipient.

In an attempt to assess the c-aAb levels in blood donors and transfusion recipients on a larger scale, we developed and validated a multiplex assay to screen for the presence of five previously described naturally occurring autoantibodies (anti-IL-1α, -IL-6, -IL-10, -IFNα and -GM–CSF autoantibodies). Here, we report the establishment and the validation of an assay and the first screening results from two groups of patients receiving blood transfusions who subsequently were observed with or without a febrile transfusion reaction.

2. Materials and methods

2.1. Plasma samples and pooled human IgG

Samples from blood donors and transfusion recipients (patients) with (N = 47) and without (N = 51) febrile reactions were obtained from the blood bank. These samples were taken 0–4 days before and 0.5–2 h after the blood transfusion. Plasma samples were stored at −20 °C and −80 °C for immediate use and for long-term storage, respectively. All of the blood donors fulfilled the European Union-Directives for blood donation criteria.

Previously, c-aAb positive blood donor plasma samples were identified by radioimmunoassay (RIA) and mini-pool screening screenings (Galle et al., 2004b; Hansen et al., 1994; Svenson et al., 1998; de Lemos et al., 2010). The pharmaceutically prepared human IgG pools for intravenous use were from HyQvia (Baxter) and Privigen (CSL Behring).

2.2. Proteins and antibodies

For the coupling (multiplex), radiolabeling, and subsequent displacement investigations, recombinant carrier-free human GM–CSF (R&D Systems), IFNα (R&D Systems), IL-1α (R&D Systems), IL-6 (R&D Systems) and IL-10 (R&D Systems) were used. Bovine serum albumin (BSA; Sigma-Aldrich) was used for the blocking of the beads. For the displacement experiments, specific polyclonal goat anti-human -GM-CSF (R&D Systems), -IL-6 (Peprotech) and -IFNα (Peprotech) -antibodies were used.

2.3. Cytokine radiolabeling

Recombinant forms of the cytokines were iodinated with the chloramine-T method and processed as previously described (de Lemos et al., 2009b). The resulting ^{125}I-labeled cytokines were repeatedly chromatographed on Sephadex G-75 (Amersham Biosciences) to yield tracers with more than 90% binding to cellular receptors, preserved bioactivity on target cells, and specific activities ranging from 50–250 cpm/pg (de Lemos et al., 2009b; Hansen et al., 1995).

2.4. Screening of the plasma samples for cytokine autoantibodies by RIA

The samples were screened for c-aAbs from mini-pools of 90 plasma samples that were adjusted to 25% (v/v) in phosphate buffered saline (PBS) that was supplemented with 0.1% (v/v) Triton X-100 (Sigma-Aldrich), 0.1% (w/v) gelatine (Sigma-Aldrich), and 2 mM EDTA (VWR – Bie & Berntsen). Then, 3500 cpm–5000 cpm ^{125}I-recombinant cytokine was added in a final volume of 200 μl. After incubation for 20 h at 4 °C, plasma IgG bound tracer and free tracer were separated with Protein G affinity chromatography and counted with a gamma counter (Hansen et al., 1994).

2.5. Cytokine coupling to microspheres

Activation of the MagPlex Microspheres (Luminex Corp.) was essentially performed as recommended by the manufacturer. Briefly, 1.25×10^6 beads were activated for 20 min with agitation at room temperature in 100 μl of 100 mM NaH_2PO_4 (Sigma-Aldrich), pH 6.2, containing 2.5 mg/ml final concentrations of N-hydroxysulfosuccinimide (Sigma-Aldrich) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (Sigma-Aldrich). The beads were washed twice with 250 μl PBS (pH 7.4) and incubated with 1, 4 or 8 μg of cytokine that was dissolved in PBS. The mixture was incubated for 2 h at room temperature with agitation, followed by one wash with PBS containing 2% BSA, 0.5% (v/v) Tween 20 and 0.05% (w/v) Sodium Azide. After the wash, the beads were blocked with PBS containing 2% BSA for 30 min with agitation at room temperature. Due to the use of a magnetic separator (Luminex Corp), insignificant amounts of the beads were lost during the coupling. The beads were stored at 4 °C in the dark until use.

2.6. Anti-cytokine autoantibody multiplex assay

Coupled beads were incubated with patient plasma that was diluted in assay buffer, which consisted of PBS containing 10% v/v citrate (Terumo BCT, to prevent plasma coagulation subsequent to sample dilution), 2% (w/v) skimmed milk powder (Merck Millipore, to promote the detection of specific cytokine-autoantibody interaction (Svenson et al., 1993), 1% (w/v) BSA, 0.5% (v/v) Tween 20, and 0.05% (w/v) Sodium Azide. Fifty microliter volumes containing a validated number of beads (varying between 1000–3000 per cytokine per well) were sealed from light and incubated with 50 μl plasma (giving a final 6- fold plasma dilution) for 1 h with agitation at room temperature. The beads were washed three times with a magnetic plate separator (Luminex Corp.). PE-conjugated goat anti-human IgG (20 μl/ml, Life Technologies) was added to each well and incubated for 30 min at room temperature with agitation, in the dark. The beads were washed 3 times before resuspension in assay buffer, read using a Luminex 100 System (Luminex Corp.) and analyzed using the StarStation software (Luminex Corp.).

2.7. Validation of the PE labeled secondary antibodies for IgG subclass sensitivity evaluation

Washed RhD positive red blood cells (RBCs) were diluted to a concentration of 2×10^8 cells/ml and incubated with previously validated human monoclonal anti-RhD IgG antibodies of all IgG subclasses (Nielsen et al., 2007). As a negative control, RhD negative RBCs were similarly treated; however, they were incubated with PBS containing 1% BSA. After incubation for 30 min at 37 °C, followed by four wash cycles with PBS for 30 s, the cells were resuspended in 100 μl of Cellstab (Bio-Rad). Subsequently, 1×10^6 RBCs were mixed with 100 μl of PBS containing 2 μl of PE-conjugated goat anti-human IgG (Life Technologies) and incubated for 30 min at room temperature in the dark. After washing, the RBCs were re-suspended in 500 μl of BD FACSFlow™ (BD Biosciences) and measured with a BD FACSCanto™ (BD Biosciences).

2.8. Intra and inter assay variation

To assess the intra- and inter-assay variation of the multiplex assay, known c-aAb negative and positive plasma samples were run five times in triplicate. The intra- and inter-assay variations were calculated as the coefficient of variation.