INRA, a new high-frequency antigen in the INDIAN (IN023) blood group system

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Abstract:

BACKGROUND: The INDIAN blood group system comprises 4 antigens sensitive to enzymes and 2-aminoethyl isothiouronium bromide (AET).

AIM: The patient's antibody was investigated for its specificity to the high-frequency antigens (HFA) of this system.

MATERIAL AND METHODS: Low ionic strength solution (LISS)-tube/LISS-indirect antiglobulin test (IAT) methods were used. The patient's red blood cells (RBCs) were tested with antisera to HFA. Her antibody was tested with RBCs lacking the HFA. Furthermore, it was tested with RBCs as untreated or treated with enzyme or AET. The genetic sequence was studied for mutation in CD44 gene that encodes INDIAN antigens.

RESULTS: The patient was grouped A1B, RhD+, antibody screening test positive, direct antiglobulin test negative. A negative autocontrol test had suggested to the alloantibody being present. Antibody had agglutinated RBCs in LISS-tube at RT and by LISS-IAT at 37°C. The RBCs of the 11-cell panel, those lacking HFA and from 50 random donors, were agglutinated by her antibody indicating its specificity to the HFA, though the RBCs of Lu (a-b-)In (Lu) type showed a weaker reaction. The patient's RBCs were agglutinated by antisera to a number of the enzyme-sensitive HFA, including those of INDIAN blood groups. The antibody showed reduced reactivity with the RBCs treated with papain, chymotrypsin, and AET but resistant to trypsin and dithiothreitol. The patient's genetic sequence revealed a novel homozygous mutation 449G>A in exon 5 of CD44.

CONCLUSION: The antibody to enzyme sensitive HFA was tested for serological and molecular genetics studies and found to be directed to the novel HFA, named as INRA of the INDIAN blood group system and was assigned a numerical symbol IN: 005 by the International Society of Blood Transfusion (ISBT).

Keywords: A new high-frequency antigen, in the INDIAN blood groups, INRA

Introduction

The INDIAN (023) blood group system currently consists of 4 antigens, namely, In(a), In(b), In3, and In4. Of these, In(a) occurs in a low frequency of about 3% in Indians.[8] It was less uncommon among the Arabs (11.8%) and Iranians (10.6%) tested.[3] The Salis antibody to a high-frequency antigen (HFA), found earlier in a Pakistani patient, was directed to the antigen that turned out to be antithetical to In(a), hence was named as In(b).[3] The INDIAN blood group system was further expanded in the year 2007 when two more antigens, namely, INFI and INJA were recognized as part of the system. Both these antigens were HFA. Of these, the INFI was detected among the Moroccans, while the INJA was found among the individuals who had their origin in the Indian subcontinent.[4] These 4 antigens of the INDIAN (023) blood group system are now known by their numerical terms IN: 001, IN: 002, IN: 003, and IN: 004 assigned by the ISBT.[3]
Antibodies to INDIAN blood group antigens result in direct agglutination of red blood cells (RBCs) in saline medium but react stronger by the indirect antiglobulin test (IAT). The antigens of the system are sensitive to proteolytic enzymes like papain and chemicals like 2-aminoethyl isothiuronium bromide (AET). Such properties may provide hints in the identification of the antibodies. We encountered such an antibody to HFA that we thought, may belong to the INDIAN blood group system, so we investigated the case from that angle.

Materials and Methods

The case
A 40-year-old female had a history of seven pregnancies but never received transfusion. She was hospitalized with continuous bleeding p.v. due to the uterine tumor. In the face of anemia, with the hemoglobin level of 8 g/dL, patient was planned for transfusion. Her blood specimen was referred to arrange blood units in case the patient required a transfusion during/after surgery. The pretransfusion compatibility tests were initiated. Antibody screening test (AST) was positive, and the cross matching with several blood units was incompatible. Hysterectomy was performed without blood transfusion. The standard serological methods were employed in pretransfusion compatibility tests and other serological workups. For molecular genetics study, the DNA was extracted from the patient’s sample. The exons of the hemopoietic isoform of the CD44 gene were amplified by polymerase chain reaction and sequenced.

Results

The patient was grouped as A1B, RhD+ with a positive AST. The direct antiglobulin test and the autocontrol tests were negative, indicating the presence of alloantibody in her serum. The antibody strongly agglutinated the RBCs in saline/low ionic strength solution (LISS) phase at room temperature and by LISS-IAT at 37°C. The antibody showed uniform hemagglutination strength on over 50 random samples and 11 commercial RBCs panel. The antibody failed to react with the RBCs pretreated with papain enzyme and the AET. In accordance with these observations, the patient’s RBCs were tested and gave positive reaction with anti-In(b), thus ruling out the specificity to this HFA. After initial serological investigations in our laboratory, her blood specimen was referred to IBGRL, UK to rule out/rule in the specificity to other known antigens of the INDIAN blood group system, namely, In3 and In4. As the antibody had reacted with red cells lacking In3 and In4 antigens, its specificity against these antigens of the INDIAN blood groups was ruled out. In addition, the antibody had reacted with RBCs of several “null” phenotypes, including Gerbich-null, Rh-null,-D/-D, MkMk, Fy(a-b-), Gy(a-), and K0, thus ruling out its specificity to the hitherto known HFAs. However, the RBCs of the Lu(a-b-)/In (Lu) type showed a depressed reactivity with the antibody. Furthermore, the antibody was not inhibited by the soluble proteins of DAF (chromer) and Yt(a) blood group antigens so its specificity to those HFAs was ruled out. The antibody did not agglutinate RBCs if they were treated with enzyme chymotrypsin, though it reacted with RBCs treated with trypsin or dithiothreitol.

The patient’s RBCs were typed positive with antisera to a number of papain-sensitive and papain-resistant HFAs, including In(b), In3, IN4, Kn(a), McC(a), Yt(a), U, Vel, En(a), Kp(b), Js(b), Wr(b), Ge, CD99. These findings had corroborated in ruling out the specificity of the patient’s alloantibody to these antigens.

The genomic analysis revealed the patient to be IN * B (IN * 02) homozygous. In exon 5 of CD44, a novel homozygous missense mutation c. 449G>A was revealed, encoding p.Arg150His amino acid substitution at the protein level. This mutation resulted in a lack of expression of a novel HFA to which we have proposed the name INRA after the patient and have obtained the ISBT numerical term as IN: 005. The INRA gene sequence has been allocated GenBank accession number KX639826. Besides this, in exon 2 of the CD44, a novel homozygous synonymous (silent) mutation c.255C>T was observed in a codon for p.His85. However, for the moment, it remains an enigmatic entity.

Discussion

The antibody to HFAs makes it difficult to find a compatible blood for transfusion. It poses a problem in identification of its serological specificity as well and also gives difficulties in finding the “antigen-negative” blood unit for transfusion. Sometimes, the properties of the corresponding antigen like sensitivity to proteolytic enzymes or certain chemicals may provide a clue to the presumptive specificity of the antibody. In the present case, the antibody showed direct agglutination of red cells in saline/LISS medium and that, it did not agglutinate the RBCs pretreated with enzyme/AET thus indicating the specificity to the HFA of the INDIAN blood group system. As a rule, an alloantibody is developed in a person who lacks the corresponding antigen. In other words, the specificity of an alloantibody is ruled out, if the antibody producer is positive for that antigen. In a similar note, if an antibody has reacted with the RBCs devoid of an antigen, the specificity to that antigen is ruled out. This maneuver in the identification of antibodies may need
an exhaustive rare red cells panel that lacks the HFAs. Besides, it requires the rare antisera to HFA to be tested. In the present study, the patient’s red cells were typed positive with antisera to the HFAs, thus ruling out the specificity of her alloantibody to those antigens. Besides, her antibody had reacted with RBCs lacking the HFAs further ruling out its specificity to hitherto known HFA.

The In(a)/In(b) antigens are weakly expressed on red cells with Lu(a-b-)/In(Lu) phenotype. The antibody in the present case showed depressed reactivity with such RBCs. This observation hinted that the antigen defined by the present case showed depressed reactivity with such RBCs. The genetic sequence studied on hemopoietic patient’s antibody may belong to the INDIAN blood group system. The name INRA, given to this antigen, was ratified as the novel antigen with its numerical term as IN005 by the ISBT.

The CD44 gene is located on chromosome 11 at position p13. The In(a)/In(b) polymorphism results from the single base change in exon 2 of CD44 gene that substitutes one amino acid, from Pro to Arg at position 46 of the CD44 protein. The common allele, In(b), has G252 and encodes Arg46 while the rare allele In(a) has C252 and encodes Pro46. This has resulted from a single mutation G > C in CD44 at position 252. A lack of IN3 and IN4 results from homozygosity for mutations encoding H85Q and T163K in the CD44 gene. In the present case, the patient lacked a novel HFA (INRA, In5) that has resulted from homozygous mutation 449G>A in exon 5 of the CD44 gene encoding an amino acid change Arg150His at the protein level.

**Conclusion**

The antibody to the enzyme sensitive hitherto unknown HFA was investigated. Serological and molecular studies have provided evidence for a novel antigen in the INDIAN (023) blood group system. The new HFA named INRA, was assigned the numerical term In: 005 by the ISBT.

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**Conflicts of interest**

There are no conflicts of interest.

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