

Bennett-Goodspeed Revisited:

Analysis of Human Leukocyte Antigens on Red Blood Cells Confirms an Undeclared Blood Group System

BACKGROUND

Human leukocyte antigens (HLA) are expressed on red blood cells (RBCs) and have been described as blood group antigens, called Bennett-Goodspeed (Bg) a, b, and c. However, the main body of literature concerning HLA on RBCs dates from a time when HLA typing and serological methods were considerably less sophisticated than today. Therefore, we decided to re-evaluate the HLA antigens that can be found on RBCs.

METHODS

Blood samples from several hundred fully HLA-typed platelet donors were screened for HLA-dependent reactivity in the indirect antiglobulin test (IAT) using a humanized recombinant pan-reactive anti-HLA class I antibody. HLA-positive RBCs were analyzed further using monoclonal antibodies, characterized anti-HLA antisera, and recombinant HLA proteins to identify the HLA allotypes that cause agglutination.

RESULTS

Bio-Rad LISS/Coombs ID cards were less sensitive in detecting anti-HLA than Grifols DG Gel (data not shown). In a preliminary statistical analysis, approximately 30% of all RBC preparations were HLA-positive (Figure 1). The strongest determinant of anti-HLA antibody positivity was the HLA-B locus (Figure 2). We were able to confirm HLA-B*07:02 as a major cause of anti-HLA antibody reactivity (Figure 3).

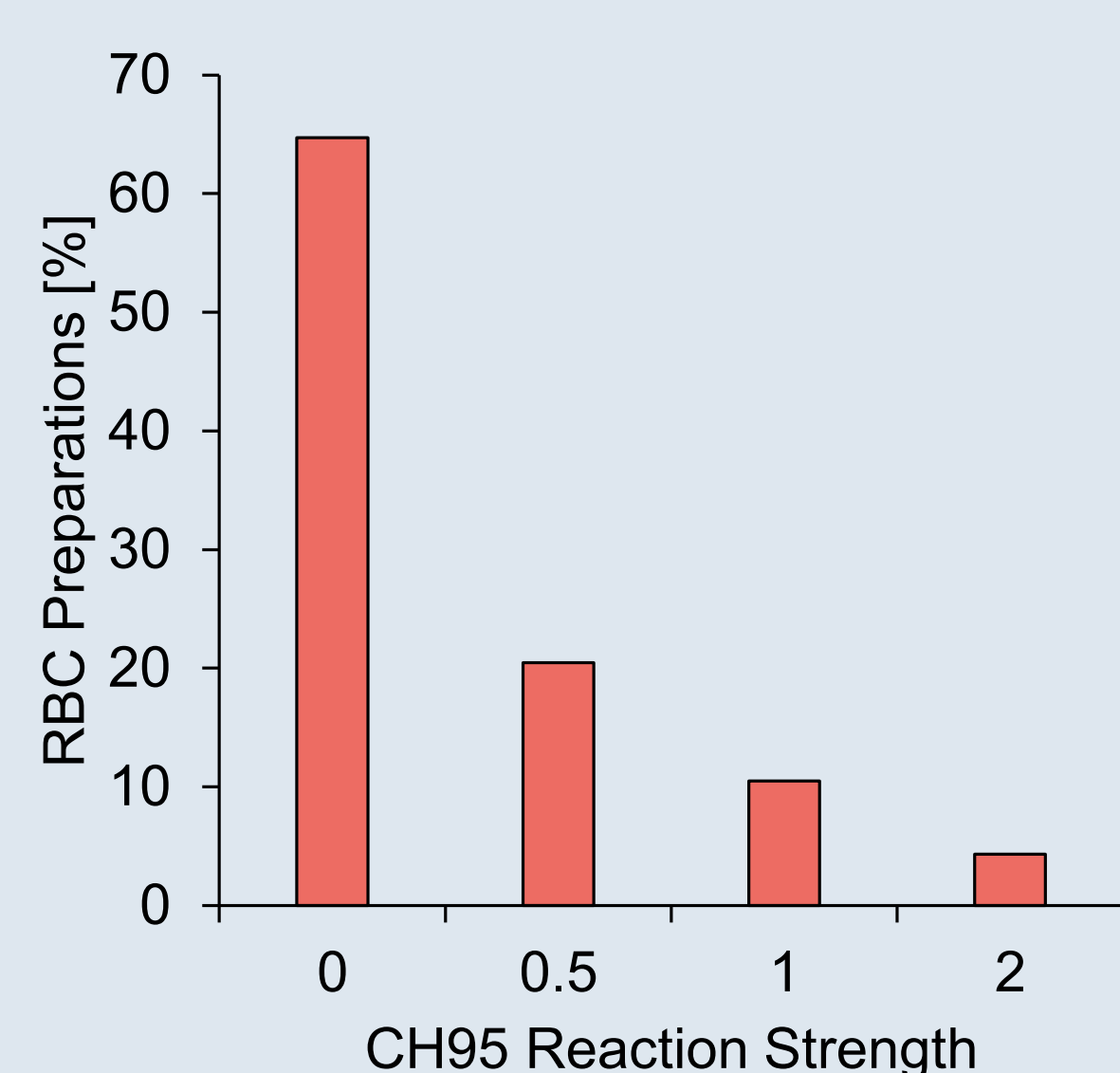


Figure 1. Reactivity of CH95, a humanized W6/32 antibody, in the IAT with RBC preparations. Given are the percentages of RBC preparations per reaction strength for all IAT results with CH95.

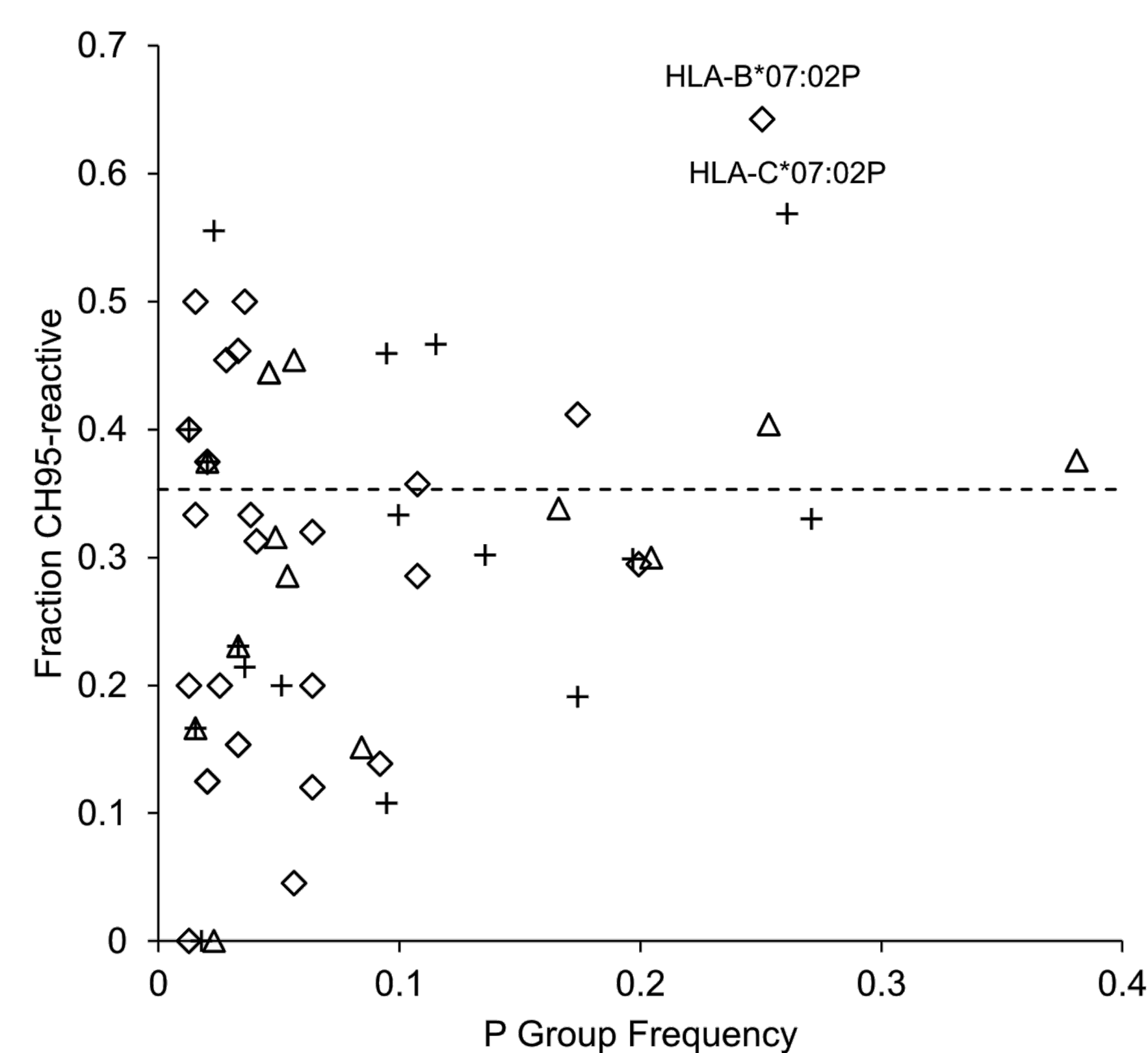


Figure 2. Fraction of CH95-positive RBC preparations vs. HLA frequency. Open triangles: HLA-A, open diamonds: HLA-B, crosses: HLA-C, dashed line: average CH95-positive fraction.

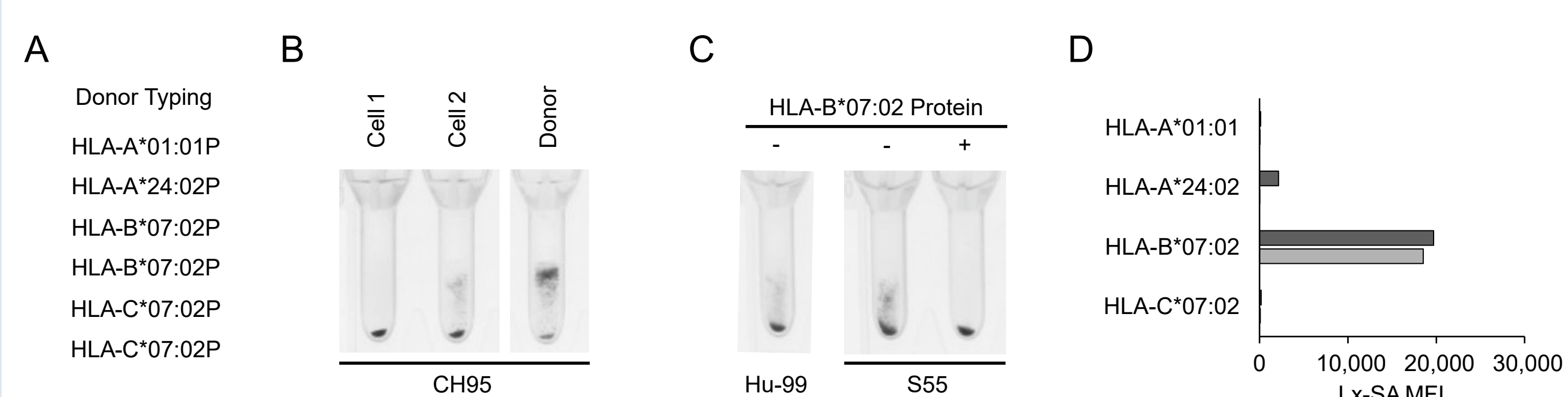


Figure 3. HLA-B*07:02 is a blood group antigen. **A:** HLA class I typing of the donor. **B:** IAT of CH95 with cell 1 (negative control), cell 2 (positive control) and donor RBC preparation (RBCP). **C:** IAT of the human monoclonal anti-HLA class I antibody Hu-99 with donor RBCP. IAT of the human antiserum S55 with donor RBCP, with and without prior addition of recombinant soluble HLA-B*07:02. **D:** MFI values from Luminex single antigen bead assays for Hu-99 (light gray) and S55 (dark gray), for the donor HLA class I.

Table 1. HLA class I fulfils the ISBT criteria for a blood group system.

ISBT Criterium	HLA Class I
The antigen(s) in the system must be found on erythrocytes	HLA Class I is found on RBCs.
The antigen(s) must be defined by human antibodies that are neither autoantibodies nor exist only as monoclonal antibodies, i.e. these antibodies have been made by at least one individual lacking the antigen in question.	S55 reacts specifically with HLA-B*07:02.
The antigen(s) must be (an) inherited character(s).	HLA Class I is inheritable.
The gene encoding the antigen(s) must have been identified and sequenced.	Gene(s) encoding HLA-B*07:02 are identified and sequenced.
The gene must be different from, and not a closely-linked homologue of, all other genes encoding antigens of existing blood group systems.	HLA class I is different from and is not a closely-linked homologue of existing blood group systems.

CONCLUSION

HLA class I expression on RBCs is widespread and **HLA class I fulfils all the criteria for a blood group system (Table 1)**. There is anecdotal evidence of transfusion reactions caused by anti-HLA antibodies, indicating that Bennett-Goodspeed is a factor in immunohematology worthy of further investigation.

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Conflict of Interest
JS, DG, and CS are employees of
imusyn GmbH & Co. KG, a company
providing soluble recombinant HLA class I.



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