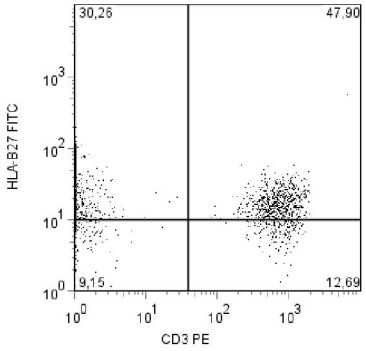

Figure 12. Flow cytometric labeling pattern of a HLA-B35/HLA-B14 patient

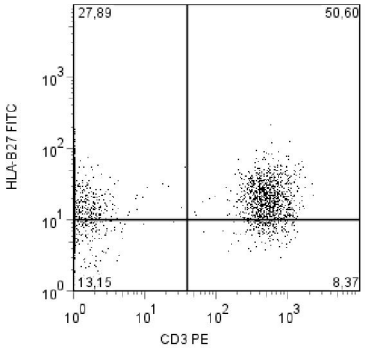

Figure 13. Flow cytometric labeling pattern of a HLA-B14/HLA-B07 patient

Taking as well into consideration the above described cases, this allowed us to understand that the anti-HLA-B07 antibody is responsible for a very strong cross-reactivity with HLA-B27, capable of masking not only HLA-B27 but also many other weaker cross-reactive HLA-B molecules, like for instance HLA-B18 (*Figure 10*).

It may well be that HLA-B35 could also behave as a cross-reactive molecule for the BD antibody since we have obtained characteristic labeling patterns in flow cytometry not only when HLA-B*35 was associated with HLA-B*37 (*Figure 11*), but also with HLA-B*14 (*Figure 12*).

However, the same weaker cross-reactivity hypothesis can be advanced regarding the HLA-B14 molecule, as in our investigation we only came across a sample where its presence was associated (and thus potentially masked) with HLA-B*07 (*Figure 13*).

Conclusions

The commercially available anti-HLA-B27 antibodies are prone to binding to various cross-reactive HLA-B molecules, generating a characteristic labeling pattern, with dots distributed, in various percentages, in both upper right and lower right quadrants. Most of these cross-reactive HLA-B molecules are belonging, as HLA-B27, to the CREG 7 group, especially HLA-B07. However, we were also able to confirm cross-reactivities produced by HLA-B molecules from other CREG groups.

Furthermore, we have shown that HLA-B18, HLA-B35 and possibly HLA-B14 are also capable of generating cross-reactive labeling patterns.

One interesting feature in flow cytometry is that the cross-reactivity produced by HLA-B07 is so strong that it can mask not only other weaker cross- reactive HLA-B molecules, but even the presence of HLA-B27.

We conclude that flow cytometry is a very useful test in screening for the presence of the HLA-B27 molecule, and cross-reactivities can be readily identified by a distinctive labeling pattern. However, we advocate that all the unclear situations should be solved by molecular HLA typing techniques.

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References

1. Brewerton DA, Caffrey M, Hart FD, James DCO, Nichols A, Sturrock RD. Ankylosing spondilytis and HL-A27. Lancet. 1973i:904-907
2. Benjamin R, Parham P. Guilt by association: HLA-

B27 and ankylosing spondilitys. Immunol Today. 1990; 11:137-142;
3. Brown MA, Pile KD, Kennedy LG, Calin A, Darke C, Bell J, et al. HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. Ann Rheum Dis. 1996; 55:266-270
4. Brewerton DA, Caffrey M, Nichols A, Walters D, Oates JK, James DC. Reiters disease and HL-A27. Lancet. 1973. 302 (7836): 996-998
5. Aho K, Ahvonen P, Lassus A, Sievers K, Tiilikainen A. HL-A27 in reactive arthritis. A study of Yersinia arthritis and Reiter's disease. Arthritis Rheum. 1974; 17:521-526
6. Orchard TR, Thiyagaraja S, Welsh KI, Wordsworth BP, Hill Gaston JS, Jewell DP. Clinical phenotype is related to HLA genotype in the peripheral arthropathies of inflammatory bowel disease. Gastroenterology. 2000; 118: 274-278;
7. Penttinen MA, Heiskanen KM, Mohapatra R, DeLay ML, Colbert RA, Sistonen L, et al. Enhanced intracellular replication of salmonella eneritidis in HLA-B27 expressing human monocytic cells. Dependency of glutamic acid at position 45 in the B pocket of HLA-B27. Arthritis Rheum. 2004; 50: 2255-2263
8. Svejgaard A, Platz P, Ryder LP. HLA and disease 1982_a survey. Immunol Rev. 1983; 70: 193-218;
9. Huhtinen M, Repo H, Laasila K, Jansson SE, Kautiainen H, Karma A, et al. Systemic inflammation and innate immune response in patients with previous anterior uveitis. Br J Ophthalmol. 2002; 86: 412-417
10. Moll JM, Harlock I, MacRae IF, Wright V. Association between ankylosing spondylitis, psoriatic arthritis, Reiter's disease, the intestinal arthropaties and Behcet's syndrome. Medicine (Baltimore). 1974; 53: 343-364
11. McGarry F, Walker R, Sturrock R, Field M. The -38.1 polymorphism in the promoter region of the tumor necrosis factor gene is associated with ankylosing spondylitis independent of HLA-B27. J Rheumatol. 1999; 26: 1110-1116
12. Lingenfelter B, Fuller TC, Hartung L, Hunter L, Wittwer C. HLA-B27 screening by flow cytometry. Cytometry (Communications in Clinical Cytometry. 1995; 22: 146-149;
13. Ward AM, Nikaein A. Comparison of monoclonal antibodies for flow cytometric analysis of HLA-B27 antigen. (Communications in Clinical Cytometry). 1995; 22: 66-69
14. Neumuller J, Schwartz DWM, Dauber E, Mayr WR. Evaluation of four monoclonal antibodies against HLA-B27 for their reliability in HLA-B27 typing with flow cytometry (FC): comparison with the classical microlymphocytotoxic text (MLCT). Cytometry (Communications in Clinical Cytometry). 1996; 26: 209-215;
15. Macardle PJ, McEvoy R, Jovanovich S. HLA-B27 expression by flow cytometry: analysis of 7 years quality assurance data. Journal of Immunological Methods. 2000; 243:51-57;
16. Levering WHBM, Wind H, Sintnicolaas K, Hooijkaas, Gratama JW. Flow cytometric HLA-B27 screening: cross-reactivity patterns of commercially available anti-HLA-B-27 monoclonal antibodies with other HLA-B antigens. Cytometry part B, 2003, 54B: 28-38
17. Hoffmann JJML, Janssen WCM. HLA-B27 phenotyping with flow cytometry: further improvement by multiple monoclonal antibodies. Clinical Chemistry. 1997; 43 (10): 1975-1981
18. Kirveskari J, Kellner H, Wuorels M, Soini H, Frankenberger B, Leirisal-Repo M, et al. False negative serological HLA-B27 typing results may be due to altered antigenic epitopes and can be detected by polymerase chain reaction. British Journal of Rheumatology. 1997; 36: 185-189
19. Glenn E. Rodey GE-HLA beyond tears. Introduction to Human Histocompatibility, second edition, 2000, chapter 2: Structure and function of the HLA Complex, Pel Freez