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Response to Comment on "Influence of HLA-C Expression Level on HIV Control"

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Abstract

Lo Monaco *et al.* propose that human leukocyte antigen E (HLA-E) and HLA-C expression levels both contribute to HIV control. The minimal, flat level of cell surface HLA-E detectable by staining with available HLA-E–specific antibodies questions a role for differential HLA-E expression in determining HIV control. Evidence remains far stronger that HLA-C expression levels as detected by the DT9 antibody specifically affect HIV control.

Lo Monaco *et al.* (1) suggest that our flow cytometry measurement of human leukocyte antigen C (HLA-C) expression could also detect HLA-E and propose an alternative interpretation of our data in which both of these antigens contribute to HIV control. However, published data indicate that HLA-E expression is poorly detected by flow cytometry of peripheral blood lymphocytes (PBLs) (2, 3). Although an effect of HLA-E on HIV control is an interesting hypothesis, one would need to provide compelling data showing that the published findings regarding HLA-E expression levels are incorrect, that HLA-E is expressed at differential levels (which is not supported by any published staining data), and that HLA-E expression level affects HIV control.

The monoclonal antibody (mAb) DT9 was originally raised against tamarin major histocompatibility complex and subsequently found to bind all HLA-C alleles (with similar affinity), only very rare HLA-A or HLA-B alleles, and the nonclassical HLA-E molecule (2, 4). We used flow cytometry with DT9 to measure HLA-C expression levels on PBLs from healthy donors (2, 5), which was justified because HLA-C is expressed at substantially higher levels than HLA-E on PBLs. Indeed, the DT9 binding that we reported correlated both with donor HLA-C allotype and reverse transcription polymerase chain reaction (RT-PCR) measurement of HLA-C transcript levels, indicating that HLA-C is the antigen primarily detected by flow cytometry of PBLs with DT9 (2, 5).

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Lo Monaco *et al.* suggest that substantial levels of HLA-E can be detected on PBLs by cytometry based on a study from Corrah *et al.* that used the mAb 3D12, which recognizes HLA-E, but the staining with mAb 3D12 to PBLs in this study was negligible, averaging less than twofold above unstained controls (3). It was necessary for the authors to perform a statistical analysis from 30 donors to conclude that the 3D12 stain was any different from the control. This is in contrast to the robust cytometry staining of PBLs that we observe with several mAbs to HLA-C. It is not certain that even the very weak 3D12 binding to PBLs represents detection of HLA-E, because some cross-reactivity with classical HLA alleles has been reported for the mAb 3D12 (6). Indeed, Corrah *et al.* proceeded to use DT9 binding to reflect differences in HLA-C expression levels between individuals (3).

We had previously compared binding of the mAb MEM-E/08 (which binds only HLA-E) and the mAb DT9 (which binds HLA-E and HLA-C) to demonstrate that flow cytometry binding of DT9 to PBLs represents detection of HLA-C (2). The mAb MEM-E/08 bound at least as well as mAb DT9 to a transfectant expressing only HLA-E, demonstrating that MEM-E/08 is at least equivalent to DT9 in affinity for HLA-E. Alternatively, staining of PBLs was evident only with the mAb DT9 and not MEM-E/08, indicating that HLA-E levels on PBLs are below the threshold of detection and that DT9 reactivity with PBLs detects predominantly HLA-C. Lo Monaco *et al.* argue that mAb MEM-E/08 recognizes only β_2m-free antigen and that HLA-E is underestimated on PBLs, but the evidence that mAb MEM-E/08 recognizes β_2m-free HLA-E specifically is based on inconclusive isoelectric focusing analyses of HLA precipitations (7). More formal demonstration of the HLA-E conformations recognized by mAb MEM-E/08 along with an analysis of the HLA-E conformations present on PBLs, rather than cell lines, is needed to rule out the more parsimonious interpretation of our experiment, which is that binding of the mAb DT9 to PBLs detects predominantly HLA-C.

The argument that HLA-E expression substantially contributes to the pattern of DT9 binding is not supported by existing data. There are no measurements of HLA-E expression levels on PBLs showing that HLA-E varies between individuals in correlation with HLA-C allotype or the rs9264942 genotype, both of which mark DT9 binding in our studies. Rather, staining with the mAb 3D12 showed no correlation with rs9264942 genotype (3). HLA-A and -B are expressed at much higher levels than HLA-C on normal PBLs, so it is predominantly these antigens that will supply the leader peptide for HLA-E expression, adding further doubt to the possibility that HLA-E and -C levels correlate.

In contrast, the evidence for variation in HLA-C expression level that affects HIV control is substantial. HLA-C levels have now been shown to vary between individuals by multiple assays (microarray, RT-PCR, and flow cytometry). One of the mechanisms determining variation in HLA-C expression level involves a polymorphic microRNA binding site in the HLA-C 3′ untranslated region, which is absent in HLA-E (8). HLA-C expression levels marked by either rs9264942 genotype (9) or mAb DT9 staining (5) correlate with the frequency of HLA-C–restricted HIV-specific cytotoxic T lymphocyte responses and viral escape mutations in vivo.

Thus, there is no evidence that HLA-E expression levels approach those of HLA-C on PBLs or that HLA-E expression levels differ across individuals to give the patterns of DT9 binding that we detect. Therefore, we interpret our observations as demonstrating that differences in expression levels of HLA-C specifically across individuals correlate with control of HIV infection.

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