Response

Absence of CCR5 intracellular pools in most CD4 and CD8 T cells

Guglielmi et al report that a minority (15%) of circulating $CD4^+$ T cells show intracellular staining with a murine monoclonal antibody, 2D7, which recognizes a domain on the second extracellular loop of CCR5. They find that this staining is not seen in circulating cells obtained from a patient homozygous for a 32 bp deletion in CCR5. As expected, modulating receptor trafficking by incubating cells with either brefeldin A (blocking transport to the cell surface) or CCL4/MIP-1β (agonist-mediated receptor down-modulation) resulted in an increase in the mean fluorescence intensity signal for intracellular CCR5 staining in CCR5-positive cells, but in each case the majority of the cells analyzed remained CCR5-negative.

In contrast to an earlier report by Achour et al[1] suggesting that all T cells express high levels of intracellular CCR5, we report[2] that most circulating blood cells do not in fact express CCR5. Our conclusion is based on the demonstration that circulating blood T cells sorted for the absence of cell-surface CCR5 had neither mRNA for CCR5 nor CCR5 protein detectable by immunoblot. Our findings are therefore compatible with those of Guglielmi et al in this key respect.

In addition, we suggest that Achour et al obtained false-positive binding signals for CCR5 resulting from the fixation/permeabilization conditions used, and show that this phenomenon could be avoided by changing the fixation/permeabilization conditions. Hence, our results are also compatible with those of Guglielmi et al in this regard.

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References

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2. Pilch-Cooper HA, Sieg SF, Hope TJ, et al. Circulating human CD4 and CD8 T cells do not have large intracellular pools of CCR5. *Blood.* 2011;118(4):1015-1019.

To the editor:

The treatment of essential thromobocytosis revisited

In "How I treat essential thrombocythemia," Beer et al[1] unfortunately chose not to use all the evidence available to them. Essential thrombocytosis (ET) and its companion myeloproliferative disorders (MPDs), polycythemia vera (PV), and primary myelofibrosis (PMF) exhibit substantial phenotypic mimicry but have different natural histories, complication rates, and treatment requirements. Thus, diagnostic accuracy is vital for therapy to be safe and effective. Much ink has been spilled over the utility of marrow