VLA-5 and transendothelial migration

To the editor—We would like to call attention to a mistake in the December 2001 article by Zou *et al.*[1], which dealt with the production of stromal cell–derived factor 1 (SDF-1) by tumor cells and the recruitment of precursor plasmacytoid dendritic cells (preDC2). This mistake appears throughout the article, in the abstract, results, figures and discussion.

It is well established in the field of cell adhesion that the major ligand of vascular cell adhesion molecule-1 (VCAM-1) is very late antigen-4 (VLA-4; also known as $\alpha_4\beta_1$, CD49d/CD29)[2] and not VLA-5 ($\alpha_5\beta_1$, CD49e/CD29)[3]. VLA-4 binds to domain 1 and 4 of VCAM-1, an immunoglobulin superfamily member induced by cytokines on endothelium[2,4]. VLA-4 also binds to an alternatively spliced domain of the extracellular matrix (ECM) protein fibronectin (CS-1), whereas VLA-5 binds to a distinct domain containing the sequence Arg-Gly-Asp[5,6].

Zou *et al.* showed that preDC2s isolated from tumor ascites expressed higher levels of VLA-5 than preDC2s isolated from blood, and that tumor ascites induced the upregulation of VLA-5 expression on preDC2s isolated from blood. The problems arise when, in an attempt to define the role of VLA-5 in preDC2 trafficking, the authors performed blocking experiments using a monoclonal antibody against VCAM-1. They report: "anti-VCAM-1 significantly inhibited preDC2 transmigration across endothelial cells, suggesting that VLA-5 was important for preDC2 tumor trafficking." On the contrary, the blocking effect of anti-VCAM-1 in the migration of preDC2s across endothelium suggests a role for VLA-4 integrins instead of VLA-5. We have analyzed the role of VLA-4 and VLA-5 integrins in monocyte chemotaxis to SDF-1 across Transwell filters coated with either purified VCAM-1 or monolayers of human umbilical-vein endothelial cells (HUVECs). We found that the VCAM-1-supported chemotaxis was not affected by anti-VLA-5, whereas it was blocked by anti-VLA-4 (Fig. 1*a*). By contrast, anti-VLA-4 and anti-VLA-5 blocked transmigration across tumor necrosis factor-α (TNF-α)-activated HUVECs (Fig. 1*b*). Both VLA-4 and VLA-5 may interact with fibronectin secreted by HUVECs into the underlying basement membrane and ECM (ref. 7), and in addition, VLA-4 can interact with VCAM-1.

The potential role of VLA-5 upregulation in DC tumor trafficking should be studied using anti-VLA-5. Similarly to monocytes, it is likely that anti-VLA-5 blocks transendothelial migration of tumor preDC2s. Moreover, the experimental model of transendothelial migration used by Zou *et al.* may not be physiologically relevant to studying the role of VLA-5 upregulation in the trafficking properties of tumor preDC2s. These cells are isolated from ascites fluids and are representative of an extravascular tissue compartment. Thus, upregulation of VLA-5 in this pool of tissue resident cells might be considered more important for cell migration through ECM than for transendothelial migration. Under physiological conditions, blood preDC2s expressing high levels of VLA-4 and low levels of VLA-5 constitute the DC pool involved in transendothelial migration. Upregulation of VLA-5 by SDF-1 would need to be rapid to affect preDC2 transendothelial migration. In this regard, it would be interesting to know the length of time needed for the upregulation of VLA-5 by tumor ascites on blood preDC2s. Originally, VLA integrins were described in leukocytes as 'very late antigens', as their expression was induced upon long-term culture[3]. Thus, it is likely that upregulation of VLA-5 occurs subsequent to DC transendothelial migration, instead of having functional relevance for transmigration.

In conclusion, it is wrong to assume a role for VLA-5 in DC trafficking based only on the blocking ability of anti-VCAM-1; VCAM-1 has not been so far demonstrated to be a ligand for VLA-5.

PALOMA SANCHEZ-MATEOS,
GONZALO DE LA ROSA &
NATIVIDAD LONGO
Laboratorio de Inmuno-oncología
Hospital General Universitario
"Gregorio Marañón"
Madrid, Spain
Email: inmunoonc@hispacom.net

Zou and Curiel reply—We thank Drs. Sanchez-Mateos, de la Rosa and Longo for their thorough analysis of our work[1]. As they point out, VCAM-1 is not a ligand for VLA-5, and therefore experiments with anti-VCAM-1 should not be used to draw conclusions regarding the potential role for VLA-5 in cell migration. Our work demonstrated that tumor-associated preDC2s expressed CD49d (VLA-4) equivalently to blood preDC2s, whereas CD49e (VLA-5) was expressed to a significantly greater degree. Further, tumor-derived SDF-1, which we implicated in tumor immunopathology, increased VLA-5 expression in blood preDC2s. Thus, our data implicated VLA-5 as a potential mediator of preDC2 adhesion/transmigration. The correct experiment, as pointed out by Sanchez-Mateos *et al.*, is to use anti-VLA-5 on preDC2s, and evaluate the effect on cell trafficking, which we have now done.

By using a similar technique[1], we are able to show that anti-VLA5 (Pharmingen San Diego, California, clone IIA1, 5 mg/ml) significantly inhibits tumor preDC2 migration in adhe-

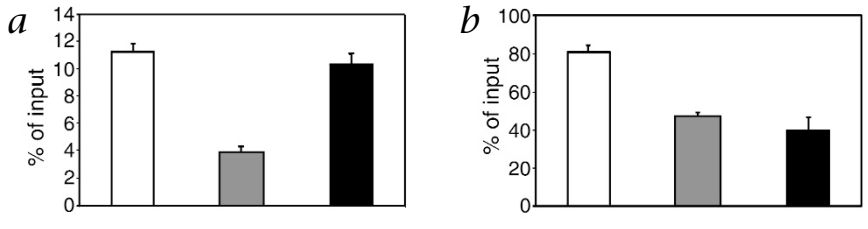

Fig. 1 Role of VLA-4 and VLA-5 in monocyte chemotaxis to SDF-1. ***a*** and ***b***, Transwell filters were coated with either (*a*) VCAM-1 (10 μg/ml) or (*b*) HUVEC monolayers treated with TNF-α to induce expression of VCAM-1. Monocytes were incubated with control W6/32 anti-HLA class I (□), anti-VLA-4 HP2/1 (■) or anti-VLA-5 IIA1 (■) (Pharmingen, San Diego, California) at 10 μg/ml for 20 min, and then added to the top of the Transwell chambers. Monocytes were allowed to migrate for 1 h across VCAM-1 and 2 h across HUVECs. Each bar represents the mean ± s.d. of 2 assays.