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Monocytes/macrophages but not T lymphocytes are the major targets of the CCL3/CCL4 chemokines produced by CD38+CD49d+ chronic lymphocytic leukaemia cells

The key role of the microenvironment for tumour growth and aggressiveness has been described for different B-cell malignancies, including chronic lymphocytic leukaemia (CLL). The dependency of CLL cells on their microenvironment is supported by several studies showing that CLL cells can be rescued from apoptosis *in vitro* if co-cultured with stromal cells, fibroblasts, dendritic or nurse-like cells. These cells are known to provide a variety of survival stimuli for CLL cells, mediated via soluble factors and extracellular matrix components, as well as direct cell-cell contact. Notably, some soluble factors are also produced by CLL cells themselves, allowing both autocrine activation of CLL cells, and paracrine recruitment of additional microenvironmental accessory cells (reviewed in Burger et al, 2009a; Caligaris-Cappio, 2003).

Recently, the two T cell chemokines C-C motif chemokine 3 (CCL3) and C-C motif chemokine 4 (CCL4) have been described to be over-produced by CLL cells upon their co-culture with nurse-like cells, as well as B cell receptor (BCR) stimulation (Burger et al, 2009b). In parallel studies by our group, we similarly described the production of CCL3 and CCL4 by CLL cells (Zucchetto et al, 2009), especially when expressing the bad prognosticators CD38 and CD49d (Shanafelt et al, 2004; Gattei et al, 2008). In particular, we observed the release of CCL3 and CCL4 by cultured CLL cells upon CD38 triggering, and found CCL3 protein in CLL cell cytoplasms from bone marrow biopsies (BMB) of CD38+CD49d+ CLL patients (Zucchetto et al, 2009).

Regarding the putative function suggested for these CLL-derived chemokines, Burger et al (2009b), although not providing direct data, extensively discussed the possibility that CCL3/CCL4 secretion by CLL cells may account for recruitment of T lymphocytes at sites of CLL infiltration, thus favouring productive CLL-T lymphocytes interactions. On the other hand, in our study, we described the presence of an abnormally high number of infiltrating CD68+ monocyte/macrophages in CLL-involved areas of BMB from CCL3/CCL4-producing CD38+CD49d+ CLL. We also showed how these monocyte/macrophages contributed to upregulate vascular cell adhesion molecule 1 (VCAM-1) expression by the stromal/endothelial component of the bone marrow (BM) microenvironment, thus promoting VCAM-1/CX49d interactions, which deliver pro-survival signals for CD49d-expressing CLL cells (Zucchetto et al, 2009).

This study investigated whether, in addition to monocyte/macrophages, other cell types could be recruited by CLL-derived CCL3/CCL4 in CLL-involved BM microenvironment. To address this issue, we first evaluated by flow cytometry the expression of CCR1 and CCR5, the CCL3/CCL4 receptors, in...
peripheral blood (PB) cell subpopulations from 14 CD38+ CD49d+ and 26 CD38−CD49d− CLL. Only PB monocytes expressed high amounts of CCR1, the specific CCL3 receptor, while low levels for CCR5, known to bind both CCL3 and CCL4 (Murphy et al, 2000), were found in all the cell types analysed (Fig 1A). Next, we evaluated the capability of PB monocytes and T lymphocytes, purified from 10 CLL expressing or not CD38 and/or CD49d, to migrate in response to CCL3 and CCL4. In keeping with the stronger expression of CCR1, monocytes actively migrated toward CCL3 also at concentrations as low as 3 ng/ml, whereas T lymphocytes required high CCL3 levels (100 ng/ml) to display slight migration capabilities (Fig 1B). Furthermore, migration toward CCL4 was negligible for both T cells and monocytes (data not shown), in agreement with the low expression of the CCL4 receptor CCR5 by all the PB cell subpopulations. Consistently, immunohistochemical analysis of BM CLL-involved areas from 16 CLL (nine CCL3−CD38−CD49d− and seven CCL3+CD38+CD49d+ cases) failed to correlate the number of infiltrating CD3+ lymphocytes with the CCL3+CD38+CD49d+ phenotype of CLL cells (Fig 1C). Conversely, in keeping with our previous report (Zucchetto et al, 2009), a higher number of infiltrating CD68+ cells was found in the context of CLL-involved areas from CCL3+CD38+CD49d+ cases. Notably, double immunostaining indicated that the CD3+ T cell component mostly lacked CCR1 expression. By contrast, CCR1 positivity appeared to be associated with stellate or spindle-shaped cells suggestive of infiltrating macrophages (Fig 1D).

According to these results, T cells do not emerge as relevant players in CCL3/CCL4-driven dynamics in CLL BM microenvironment. Rather, the CCL3/CCL4 chemokines, CCL3 more effectively than CCL4, preferentially target monocytes/macrophages, which are recruited by this/these chemokine/s, and appear increased in the context of microenvironmental sites of CCL3/CCL4-producing CLL. Nevertheless, T cells are

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constantly present in CLL-involved areas and may still have a role in CLL cells survival and growth (Ghia et al., 2002; Burger et al., 2009a), although outside the events driven by the overproduction of CCL3/CCL4 by CLL cells.

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Hairy cell leukaemia-variant and splenic red pulp lymphoma: a single entity?

In a recent correspondence article, Hockley et al (2010) reported that the hairy-cell lymphoproliferative disorders display distinct Immunoglobulin Gene Heavy Variable (IGHV) repertoires. Notably, it was suggested that the hairy cell leukaemia-variant (HCL-V) repertoire, analysed for the first time for IGHV mutational status, shares more similarities with splenic marginal zone lymphoma/splenic lymphoma with villous lymphocytes (SMZL/SLVL) than with hairy cell leukaemia (HCL) (Hockley et al., 2010). We agree with the authors position, who then called into question the origin of