NK cell function, we cultured NK cells overnight with 1000 IU/mL of IL-2 in the presence/absence of 15 μM CADO. We observed that CADO significantly suppresses IL-2 induced tumor necrosis factor-α and interferon-γ production by NK cells (Figure 1C-D). We also studied the effect of overnight CADO treatment on unstimulated NK cells. When these CADO pretreated NK cells were exposed to K562 leukemic cells, the former exhibited significantly reduced degranulation capacity compared with NK cells cultured without CADO (Figure 1E).

Finally, using mass spectroscopy, we could demonstrate that up-regulation of ectonucleotidase CD73 on NK cells is also accompanied by increased enzyme activity (Protocol as described in supplemental Material 1). First, we could document increased expression of CD73 on NK cells precultivated with MSCs (Figure 1F). Second, we could directly establish that NK cells precultivated with MSCs were able to produce significantly higher amounts of ADO compared with NK cells cultured alone (Figure 1G).

MSCs have the ability to home to sites of inflammation in vivo.6,7 NK cells interacting with these MSCs are likely to acquire this CD73+ NK cells have the potential to regulate NK cell activation in an autocrine or paracrine manner. This might have widespread implications for immunomodulation in the inflammatory microenvironment.

To the editor:

The FIP1L1-PDGFRA fusion gene and the KIT D816V mutation are coexisting in a small subset of myeloid/lymphoid neoplasms with eosinophilia

Spindle-shaped tryptase+/CD25+/CD117+/CD2+/- mast cells (MCs) are a hallmark of the bone marrow (BM) in myeloid/lymphoid neoplasms harboring a FIP1L1-PDGFRA (FP) fusion gene.1,2 These neoplasms that may present as chronic eosinophilic leukemia (CEL), acute myeloid leukemia/blast phase, myeloid sarcoma, or lymphoblastic lymphoma/leukemia rarely fulfill the criteria of the World Health Organization category “systemic mastocytosis with associated haematological non-mast cell disorders” (SM-AHNMD).1-5 Among 123 SM-AHNMD patients, only 12 carried an FP fusion gene.5 KIT D816V analysis performed on 8 of these FP-positive patients and on 2 cases included in another study were negative.5,6 Thus, the relationship between MCs in FP-positive CEL and KIT D816V-positive SM remains elusive.

We microdissected tryptase+ MCs from formalin-fixed, EDTA-decalcified, paraffin-embedded BM trephine biopsies of 19 FP-positive CEL patients using a PALM MicroBeam (Carl Zeiss Microscopy, Oberkochen, Germany). DNA was extracted from 100 to 250 pooled MCs per biopsy (QIAamp DNA Mini Kit; Qiagen, Hilden, Germany). The online version of this article contains a data supplement.

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References

Figure 1. **FP-positive CEL with concomitant KIT D816V mutation.** (A) Hypercellular BM biopsy with a marked increase in eosinophils (Giemsa stain). (B-E) Phenotypically aberrant MCs are arranged in loose clusters and as interstitial infiltrates (B) tryptase, (C) CD117, (D) CD2, and (E) CD25. (F-I) fluorescence in situ hybridization with a 4q12 tri-color rearrangement probe (F) alone or (G-H) combined with tryptase IH (fiction) (note nuclei with green/aqua fusion signals [arrows] as a surrogate for CHIC2 deletion). Normal tricolor green/orange/aqua fusion signals are marked by an arrowhead. (J) c-kit antisense sequence of microdissected MCs from FIP1L1-PDGFRα–positive CEL showing a KIT D816V mutation. (A-E) Photomicrographs by Horn Imaging Camera (Aalen, Germany) adopted to a Zeiss Imager.M1 (Carl Zeiss, Oberkochen, Germany) microscope. (F-I) Imaging system ApoTome.2 (Carl Zeiss).
the MCs of 5 of 19 FP-positive CEL patients (Figure 1). The KIT D816V mutation was not detectable in DNA/RNA extracted from whole white blood cells derived from peripheral blood or BM samples, probably as a consequence of the very low burden of mutated KIT D816V-positive cells, which was only detectable in DNA obtained from microdissected MCs. Fluorescence Immunophenotyping and Interphase Cytogenetic as a Tool for Investigation Of Neoplasia (FICTION) using a Vysis 4q12 tri-color rearrangement fluorescence in situ hybridization probe kit (Abbott Molecular, Wiesbaden, Germany) resulted in 1 green/aqua fusion signal with a deletion of the orange signal of the CHIC2 gene in the nuclei of tryptase-positive BM MCs in a patient with KIT D816V-positive MCs (Figure 1). The presence of CD25-positive MCs with concomitant KIT D816V and serum tryptase levels >20 ng/mL support the diagnosis of SM-AHNMD/SF-FP-positive CEL in 2 patients. This discrepancy to the previously published negative KIT D816V mutational analysis of microdissected MCs may be explained by a sampling effect because only 2 FP-positive SM-CEL cases were examined.6 It remains elusive whether FP and KIT D816V are present in the same clone or whether there are 2 separate clones. We reported on the heterogeneity of molecular aberrations in KIT D816V-positive SM with ≥1 additional mutation, for example, TET2, SRSF2, ASXL1, and others in 24 of 27 patients.7 In a murine model, expansion of eosinophils and MCs may result from an interaction between FP, interleukin 5, the ligand stem cell factor, and KIT in the absence of a KIT mutation.8 It can be speculated that the FP fusion gene favors secondary KIT mutations in MCs via growth and proliferation signals or that a yet unknown mechanism causes genomic instability with independent evolution of FP and KIT D816V.

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References


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To the editor:

Akt is essential to induce NADPH-dependent NETosis and to switch the neutrophil death to apoptosis

Neutrophil extracellular traps (NETs) have been recently identified as major contributors of several hematological and vascular diseases. These disorders include thrombosis, small vessel vasculitis, systemic lupus erythematosus, autoimmunity, pneumonia, sepsis, and blood transfusion–related acute lung injury.14 NETs are DNA-based extracellular traps that not only trap and kill invading microbes but also...
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