Response

Homing defect in hematopoietic cells from Fanconi anemia patients

We thank O’Neill and colleagues for their comments on our paper.1 We agree that assays of clonogenicity and hematopoietic repopulation are not sufficient for defining homing. However, we argue that CFC (colony-forming cell) homing assay is an acceptable in vivo approach to assess the homing capacity of human hematopoietic progenitor cells in a mouse bone marrow (BM) transplantation model.2,3

We also share the concern of the authors of the letter that results with whole BM cells may not reflect stem and progenitor cell behavior. We must point out that with current technology, it is impossible to conduct the described experiments using purified hematopoietic stem/progenitor (HSC/P) cells from Fanconi anemia (FA) patients, simply because such immature stem cells are rare in FA patients. Indeed, previous studies from our institute with 54 FA patients evaluated in the Fanconi Anemia Comprehensive Care Center at Cincinnati Children’s Hospital Medical Center show that FA patients had a 2-fold reduction in BM cellularity and a 6-fold reduction in CD34+ cell content compared with healthy adults.4 Given the inability to collect quantities of HSC/P cells sufficient to conduct the experiments described in our paper, we have instead used BM mononuclear cells as the initial input of progenitor cells from FA BM. We have determined nonobese diabetic/severe combined immunodeficient (NOD/SCID) BM homing of human progenitors by 2 different assays: progenitor clonogenic (CFC homing) assay and human CD45/CD34 flow cytometric analysis of human progenitors homed in the BM of NOD/SCID recipients. Although these 2 methodologies are very different, they serve to be mutually confirmative, thus validating the reliability of the assays and accuracy of the data.

We are pleased to learn that the authors of the letter have confirmed our adhesion results in their Fancc–/– mouse model. It is true that some of the results were obtained from Epstein-Barr virus (EBV)-transformed lymphoblasts. However, it should be noted that our control lymphoblast cells from normal donors are also EBV transformed. In addition, we showed decreased cdc42 activity in lymphoblasts derived from FA-C and FA-G patients (see Figure S1 in Zhang et al1). Thus, we do not agree with the authors of the letter that the difference in cdc42 activity is due to differential responses by FA cells to EBV immortalization.

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References


To the editor:

Nuclear factor-κB is not essential for NADPH oxidase activity in neutrophils from anhidrotic ectodermal dysplasia patients

Patients suffering from anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) fail to activate the canonical nuclear factor-κB (NF-κB) pathway due to mutations in the IKKG (inhibitory κB kinase gamma) gene encoding IKKγ, also known as NEMO (NF-κB essential modulator).1 This results in a combined cellular and humoral immune defect. In a recent study by Luengo-Blanco et al, the authors conclude that NF-κB activity is required for the transcription of the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase genes CYBB and NCF1, encoding gp91phox and p47phox, respectively.2 Both proteins are essential components of the NADPH-oxidase complex, and in patients with chronic granulomatous disease (CGD) failure to express these proteins results in a severe immunodeficiency.3 Luengo-Blanco et al base their conclusions on studies with Epstein-Barr virus (EBV)-transformed B-cell lines from patients diagnosed with EDA-ID as well as studies with pharmacologic inhibitors of NF-κB activation in the monocytic cell line U937 and repressor transfected U937 cells. In these model systems, expression of gp91phox and p47phox was severely impaired, resulting in a failure to activate the NADPH oxidase upon stimulation with phorbol 12-myristate 13-acetate (PMA), similar to EBV-transformed B cell lines from CGD patients.2

Recently, we had the opportunity to perform functional tests with primary neutrophils from 3 different EDA-ID patients. The patients were 1, 2 and 5 years of age at the time of investigation. The second patient was the brother of the third. All 3 patients had variable degrees of dermatitis and minor peripheral lymphadenopathy since birth, as well as recurrent bacterial infections, and had the pale, sparse hair and conical incisors typical for EDA-ID patients.3 The genetic defects were all identified in the IKKG gene resulting in a stop codon (p.Q365X) in exon 9 (c.1093C>T) in one patient and a missense mutation (p.Q205P) in exon 5 (c.614A>C) in the other 2 patients. NF-κB activation was severely impaired in all 3 patients, as demonstrated in Figure 1A by a significantly reduced production of interleukin-8 (IL-8), when determined by ELISA after overnight stimulation of the isolated neutrophils with lipopolysaccharide (LPS,
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