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The Early Stage of Friend Virus Erythroleukemias: Mechanisms Underlying BPA-"independent" In Vitro Growth of BFU-E*

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The erythroleukemias induced by Friend virus (FV) are characterized by a stepwise progression toward malignancy. In the *first stage*, i.e., the initial 3 weeks postinfection (p.i.), the mice show a pronounced wave of erythroblastosis in spleen, marrow, and blood [5]. Cell lines could not be established from these animals [12]. In the *second stage* (i.e., after 21 days p.i.) overt malignancy develops, as permanent cell lines can be easily obtained [12]. These are indistinguishable from the original Friend erythroleukemia lines [5].

At least two strains of FV are presently known. The first one (FVA) induces an erythroleukemia with splenomegaly, enhanced but ineffective erythropoiesis, and mild anemia [4]. The second one (FVP) causes the same type of leukemia, associated with effective erythropoiesis and marked polycytemia [13].

An *onc* gene has been demonstrated in "acute- type" RNA tumor viruses [7], but not so far in the FV complex. Indeed, the genomes of FVP and FVA consist of two components: a replication-defective spleen focus-forming virus ($SFFV_P$ and $SFFV_A$, respectively) and a replication-competent murine leukemia virus ($F\text{-}MuLV_P$ and $F\text{-}MuLV_A$, respectively) [20]. The two components have been recently cloned [9, 14, 15]. Injection of $F\text{-}MuLV_P$ or $F\text{-}MuLV_A$ into newborn Balb/c or NIH/swiss mice induces an erythroleukemia with splenomegaly and anemia [11, 14, 15]. Treatment with cloned $SFFV_P$ or its *"env"* fragment with LTR, in association with any of different helper viruses, induces in newborn and adult susceptible animals an erythroleukemia with splenomegaly, erythroblastosis, and polycythemia [10]. In contrast, $SFFV_A$ has little or no biological activity, perhaps due to defective glycosilation of its gp52 marker [19].

Our studies have been focused on the kinetics of early (BFU-E of *primitive* type) and late (CFU-E) erythroid progenitors in the *first* stage after FVP and FVA infection. In this regard, the kinetics of normal hemopoietic progenitors is controlled by specific hemopoietins. In vitro cycling and differentiation of BFU-E is largely modulated by burst-promoting activity (BPA), i.e., a glycoprotein factor of ~24,000 daltons [8, 21, 22]. CFU-E kinetics is largely regulated by erythropoietin (Ep) [6]. Proliferation of granulomacrophage progenitors (CFU-GM) is modulated by colony-stimulating factors (CSF) [2].

The first stage of FV erythroleukemia is characterized by marked amplification of the splenic pool of BFU-E [18] and CFU-E [16]. The cycling activity of the former progenitors is markedly enhanced [18]. In vitro growth of CFU-E from mice treated with FVP does not require Ep addition [3, 16, 18].

The enhanced cycling and perturbed kinetics of BFU-E in FV mice are compatible with a rise of BPA. Results obtained in our laboratory indicate that this elevation occurs. Indeed, in vitro growth of spleen BFU-E from 1-, 2-, and 3-week infected animals is partially or totally independent

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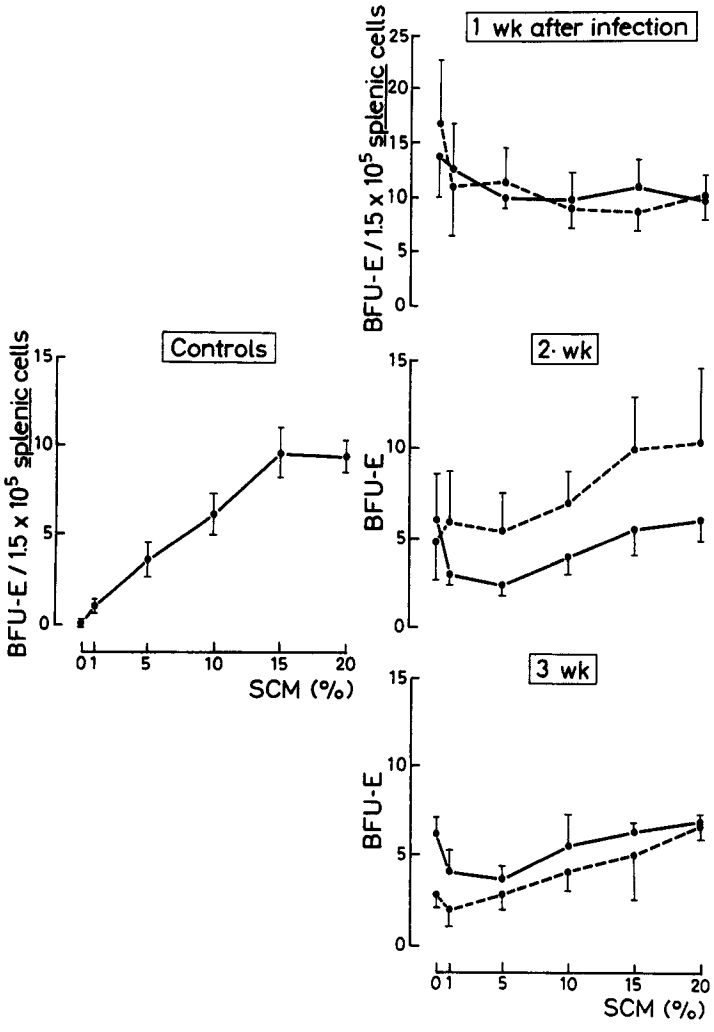

Fig. 1. SCM (see text) dose/response curve in 4% fetal calf serum cultures [8] of BFU-E from spleen of normal (controls) and 1-, 2-, 3-week infected FVP (——) or FVA (– – –) mice. Mean ± SEM values are presented (three to six experiments/points, two plates/group in each experiment)

of exogenous BPA (i.e., lectin-stimulated spleen-conditioned medium, SCM) (Fig. 1). This hormone "independence" is less clearly expressed at marrow level (Fig. 2 and data not shown). In contrast, cloning of CFU-GM from infected mice strictly requires CSF addition ([17], and results not presented here).

The "BPA independence" of BFU-E is apparently due to an in vitro rise of BPA, in turn mediated via two synergistic mechanisms: (1) hypersensitivity of BFU-E to BPA, as suggested by SCM dose-response curves for marrow BFU-E (Fig. 2). (2) Increased BPA release in culture. This is suggested by nonlinearity of cell/colony regression of BFU-E in absence of SCM (Peschle et al., in preparation), and increased BPA in medium conditioned by splenocytes from FVP-infected animals as compared to appropriate controls (Peschle et al., in preparation).

Growth of CFU-E from FVP-treated mice in serum-free cultures is largely independent of exogenous Ep [17], as previously reported. Indeed, these progenitors show marked hypersensitivity to Ep, up to independence of it (Peschle et al., in preparation). It is tentatively postulated that the rise of BPA, particularly in spleen, may play a key pathogenetic role in the early stage of FV erythroleukemias. Indeed, FV causes both (a) a rise of BPA and (b) erythroblastosis progressing into erythroleukemia. These two phenomena may either develop in parallel, or be linked by a cause/effect relationship. In the latter hypothesis the following aspects are important. Elevated BPA induces enhanced cycling of BFU-E [22]. Additionally, a glycoprotein possibly identical to BPA triggers proliferation of CFU-S [22]. The cycling BFU-E is considered a suitable target for infection and transformation by FV [1]. On this basis, the following sequence of events may hypothetically underlie FV erythroleukemias: (1) FV enhances production of BPA, particulary in the spleen. (2) Ele-

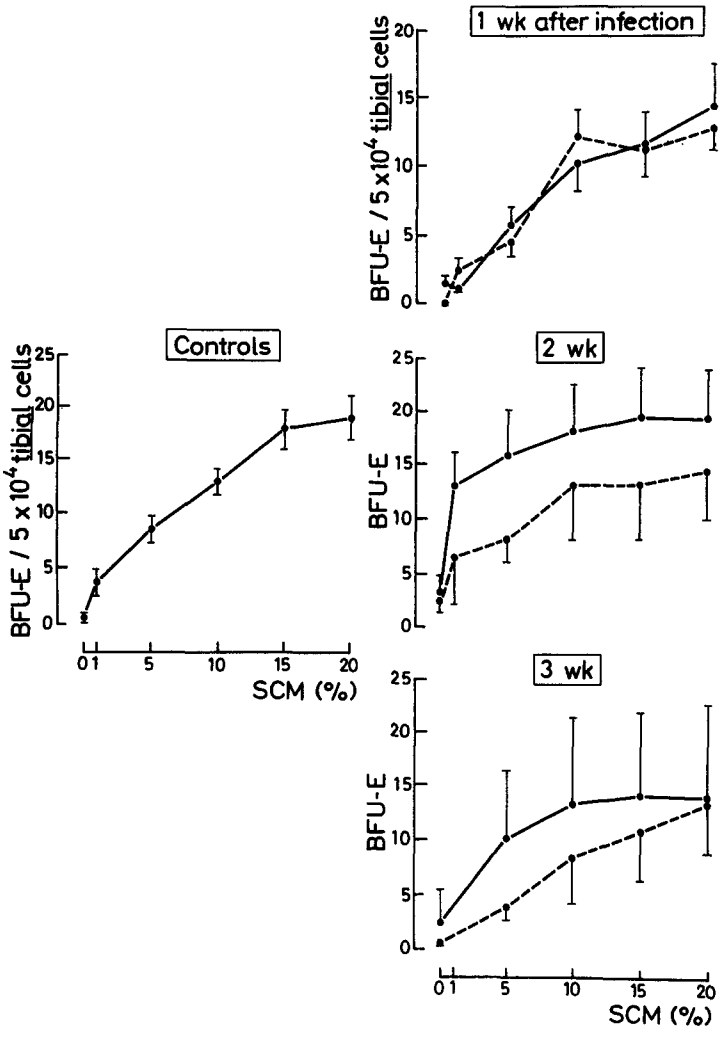

Fig. 2. SCM dose/response curve of tibial BFU-E (for details see Fig. 1): BPA hypersensitivity of BFU-E from 2- and 3-week infected FVP animals and possibly 1- and 2-week infected FVA mice

vated BPA forces quiescent BFU-E (and possibly CFU-S) into enhanced cycling, thus favoring their infection, which in turn causes BPA hypersensitivity and eventually leukemic transformation. (3) The leukemia is characterized by prevalent erythropoietic expression, due to the sustained elevation of BPA, via both extrinsic (enhanced release) and intrinsic (increased sensitivity) mechanisms. In FVP animals the erythropoietic component is effective, due to Ep hypersensitivity (up to independence) of infected CFU-E.

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