Isolation and Characterisation of a Myeloid Leukaemia Inducing Strain of Feline Leukaemia Virus

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A. Introduction

Feline leukaemia virus (FeLV) is the aetiological agent of a wide range of neoplastic and degenerative conditions [1]. The predominant, naturally occurring, FeLV-induced tumours are T-cell lymphomas, and recently some of the viral events in their pathogenesis have been elucidated. Both transduction and insertional mutagenesis of the *myc* gene are frequent concomitants of T-cell transformation by FeLV. In addition, a possible role for the T-cell antigen receptor gene in leukaemogenesis has been revealed with the discovery of an FeLV-mediated transduction of the β-chain of the T-cell antigen receptor [2, 3].

In contrast, the viral aetiology of myeloid leukaemia has received less attention. In these studies we have isolated a virus complex, FeLV-GM1 from a naturally occurring case of myeloid leukaemia corresponding to stage M6 in the FAB classification. This isolate contained both subgroups A and B of FeLV, and on passage into kittens it produced a spectrum of myeloproliferative disease including myeloid leukaemia. In vitro colony assays of bone marrow early erythroid precursor (BFU-E) and granulocytic macrophage precursor cells (GM-CFC) indicated that two stages in the development of disease could be recognised. In the first stage, in which no histopathological abnormalities were observed, there was a gross expansion in the GM-CFC compartment. At a later stage in those cats that developed myeloid leukaemia, large numbers of small clusters were superimposed on a residual normal GM-CFC colony pattern.

FeLV-GM1 contains both subgroup A and B components which were not separable by endpoint titration. Molecular analysis and cloning of the FeLV-GM1 isolate has revealed that the subgroup B component is defective for replication, and from recent pathogenesis experiments with cloned viruses it is now evident that B component was not required to induce the early proliferative events. However, only in cats inoculated with both components has full leukaemia development been observed so far.

We hypothesise that the viral events leading to myeloid leukaemia can be divided into two discrete stages. In the first stage the virus induces a polyclonal expansion of myeloid precursor cells with altered response to, and/or production of, growth factors. This proliferating cell population may now become a target for further viral events necessary for complete transformation.

B. Cellular Events in FeLV-GM1 Leukaemia

Initial experiments with FeLV-GM1 involved the passage of virus from the original tumour into newborn kittens. Of 30 cats challenged with this virus 6 died of myeloid leukaemia within 8–40 weeks, 17 developed aplastic anaemia and 7 remained clinically normal for 1 year. In order to characterise the effects of the

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Table 1. Colony-forming units of bone marrow precursor cells in control and FeLV-GM1 infected cats

Cat no.	Status	Weeks after infection	GM-CFC per 10^5 bone marrow cells	GM-CFC colony morphology	Pathology
1	Control	1.5	65	Normal	Normal
2	Infected	1.5	201	Normal	Normal
3	Infected	1.5	360	Normal	Normal
4	Control	4	48 ± 4	Normal	Normal
5	Infected	4	6 ± 2	Normal	Preleukaemic
6	Infected	4	42 ± 6	Normal	Preleukaemic
7	Control	5	57 ± 5	Normal	Normal
8	Infected	5	30	Plus 10^3 clusters	Myeloid Leukaemia
9	Infected	5	200	Plus 10^3 clusters	Myeloid Leukaemia
10	Control	8	79 ± 11	Normal	Normal
11	Infected	8	13 ± 2	Plus 10^3 clusters	Myeloid Leukaemia
12	Infected	8	ND	ND	Myeloid Leukaemia
13	Control	20	172 ± 7	Normal	Normal
14	Infected	20	792 ± 76	Normal	Normal
15	Infected	20	764 ± 11	Normal	Normal

Bone marrow from cats infected with FeLV-GM1 was prepared, plated at densities ranging from 10^4 to 10^5, and assayed as described elsewhere [7]. Results are expressed as the means ± SD of four plates; results without standard deviations are the means of two wells.

virus on bone marrow precursor cells, ten neonatal kittens were infected with ca. 10^4 ffu/ml FeLV-GM1. Starting at 10 days post-infection and at intervals thereafter infected cats with their age-matched uninfected controls were sampled for the assay of plasma viraemia and bone marrow colony-forming cells (Table 1).

Appropriate tissue was examined histologically so that correlations could be made between the extent of the disease and the pattern of myeloid colony formation (Table 1 and Fig. 1). As early as 10 days after infection a dramatic effect on the myeloid lineage was noted with a three- to six-fold increase in the number of GM-CFC colonies. At this stage both the morphology of the colonies and the histology of the marrow remained normal. In cats examined at 5 and 8 weeks after infection, myeloid leukaemia was present. Three of these cats had myeloid leukaemia with little differentiation, typical of stage M1 disease in the FAB classification, while a further cat showed clear evidence of stage 4, myelomonocytic leukaemia. A striking feature of the GM-CFC colony pattern in all of the leukaemia cats examined was the presence of several thousand small cell clusters which were superimposed on a pattern of few remaining colonies, a finding which has been recorded in some human patients with acute myeloblastic leukaemia and which is pathognomic for that disease [4]. In one of the leukaemic cats the morphologically normal GM-CFC population was expanded about four times above the control value while in two others (cats 14 and 17) the GM-CFC colonies were reduced below control values. The likeliest interpretation of these findings is that, in the first stage of the disease the virus induces an expansion of the GM-CFC population which is eventually replaced by a leukaemic population arising from these cells.

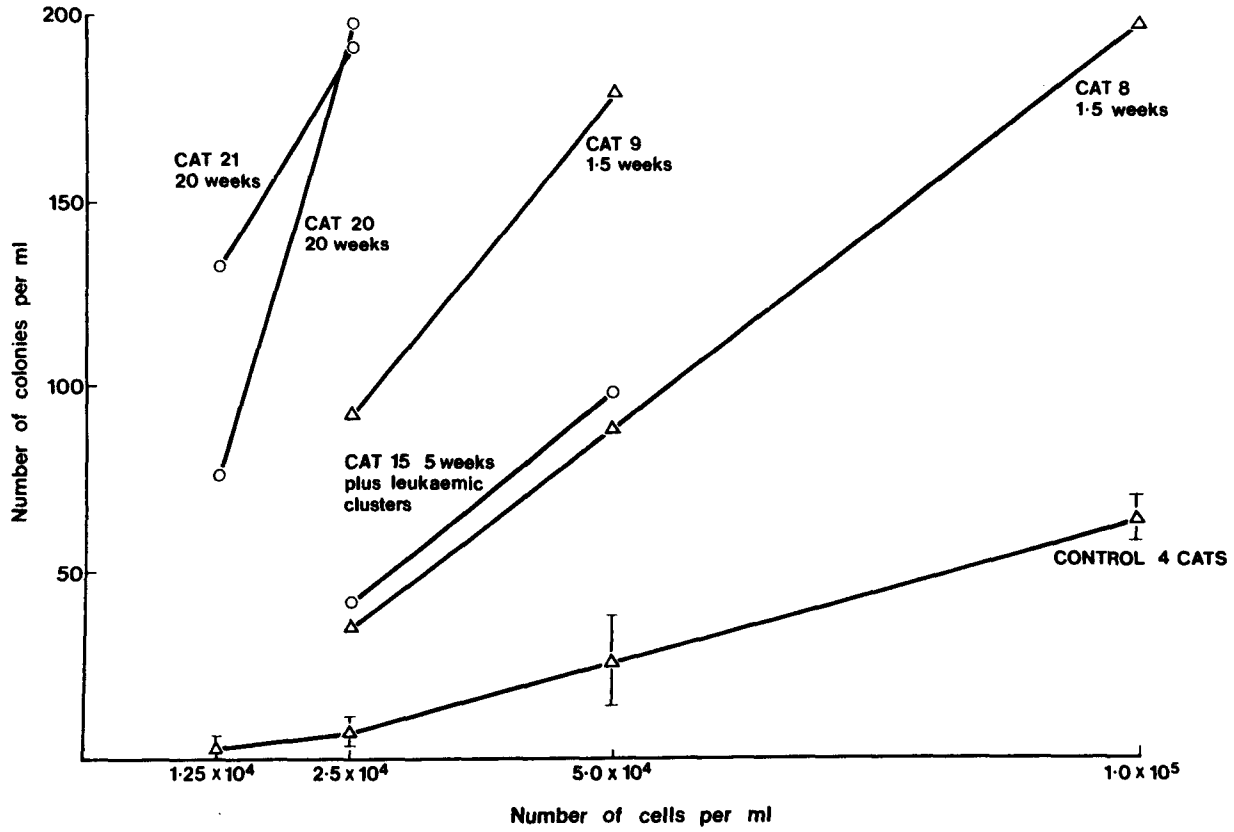

Fig. 1. Colony-forming units of granulocyte macrophage precursors (GM-CFC) determined at varying cell densities of nucleated bone marrow cells in the absence of exogenous colony-stimulating factor. The control values are the means of four cats (± SE). Only colonies with normal morphology were scored. Note in this figure cats 8, 9, 15 and 20 correspond to cats 2, 3, 9, 14 and 15 in table 1

C. Characterisation of FeLV-GM1 Virus

A distinguishing feature of cats that developed leukaemia following FeLV-GM1 infection was the presence of both subgroups A and B in the plasma. Cats that remained clinically normal were usually viraemic with subgroup A virus, while remaining latently infected with subgroup B. A temporal association between the onset of subgroup B viraemia and leukaemia was also observed. In one cat 9 months post-infection GM-CFC colony numbers remained within normal limits, and the cat was viraemic with subgroup A alone. A month later the cat had developed myeloid leukaemia and was viraemic with both subgroups. However this association is complex in that cats viraemic with subgroup A alone often had titres of less than 10^3 ffu/ml, whereas cats viraemic with both subgroups had titres of ca. 10^5 ffu/ml. Consequently the presence of the B subgroup in plasma could have been a consequence of efficient subgroup A replication rescuing the B virus from latency.

In order to resolve the roles of the individual components of FeLV-GM1 in disease induction, we undertook to molecularly clone and analyse the biological effects of the subgroup A virus alone and the reconstituted A plus B virus complex. The subgroup B virus was found to have a 1.5-kb deletion with *gag-pol* and was therefore defective for replication.

In Table 2 the result of GM-CFC colony assays plated at limiting dilution without exogenous colony-stimulating factor are presented for cats infected with either subgroup A or subgroup AB. All the AB infected cats displayed an expansion of GM-CFC at 4 weeks, as did three of the six subgroup A infected cats, indicating that the subgroup B virus was not an absolute requirement for this event.

At 38 weeks cat 22 developed myeloid leukaemia and, as in other leukaemic

Table 2. GM-CFC colony formation at limiting dilution 4 weeks after infection

Cat no.	Virus challenge	GM-CFC per 10^4 BM cells without exogenous CSF
16	Control	0.7±1.1
17	AB	10 ±4
18	AB	12 ±1
19	AB	11 ±3
20	AB	14 ±1
21	AB	18 ±4
22	AB	16 ±3
23	AB	15 ±4
24	Control	1 ±1
25	A	2 ±0
26	A	1 ±1
27	A	3 ±1
28	A	20 ±3
29	A	8 ±4
30	A	16 ±6

GM-CFC colony numbers of uninfected control cats and cats infected with molecularly cloned FeLV-GM1. Cells were plated at limiting dilution without the addition of exogenous CSF.

cats, a clonal proviral integration pattern was observed.

D. Discussion

The study of retrovirus-induced leukaemias has been of value in revealing the multistage pattern of leukaemogenesis and in implicating specific cellular genes in haemopoietic transformation. In long-term bone marrow cultures Friend virus infection permits the establishment of autonomously proliferating cell lines which may grow independently of exogenous growth factors but respond to differentiation factors and are non-leukaemic in vivo. At a later stage cells with only a limited capacity to differentiate develop and these cells are leukaemic in vivo and are often aneuploid [5]. These observations are parallelled by the in vivo experiments with FeLV-GM1 in which the initial step appears to be an increase in granulocyte macrophage cells which have an altered response to CSF. This in turn is followed by the development of leukaemic cells which do not differentiate normally in response to CSF.

The mechanism responsible for the initial GM-CFC expansion is not clear. Both the rapidity of the expansion and the absence of a clonal retroviral integration pattern suggests that this process is polyclonal. Retrovirus infection has been associated with enhanced CSF production [6], and FeLV infection of feline embryo cells can induce the production of factors with burst promoting activity (J. Abkowitz, personal communication). However, preliminary experiments in which marrow from infected cats was used as a source of CSF indicate that a minimal increase in CSF production occurs in FeLV-GM1 infection. A hypothesis worth further investigation is that infection of bone marrow precursor cells can lead to autocrine stimulation of GM-CFC.

Studies with the molecularly cloned viruses indicted that the subgroup A component of FeLV-GM1 could reproduce the early proliferation of GM-CFC. Similarly we have previously shown that another subgroup A virus, FeLV-Glasgow/1, can produce a lesser but significant increase in GM-CFC numbers soon after infection [7]. FeLV-Glasgow/1 is less rapidly oncogenic than FeLV-GM1 but has produced myeloid leukaemia in some cases.

In those cats that developed myeloid leukaemia a clonal pattern of proviral integration was observed in the bone marrow. In Friend virus-induced myeloblastic leukaemias three distinct proviral integration sites, the *fim* loci, have been identified, one of which, *fim*-2, spans the 5′ end of the c-*fms* gene [8]. In the leukaemic cats we have not detected rearrangement of the c-*fms* gene, but we cannot preclude a proviral integration some distance from this locus.

The role of the subgroup B virus in the secondary leukaemogenic events remains unresolved. One possibility is that it is

involved in overcoming viral interference in preleukaemic cells that are already infected with subgroup A [9]. FeLV subgroups are defined by viral interference so that a cell infected by subgroup A cannot be superinfected with the same subgroup but is susceptible to subgroup B infection. Consequently the initial events may be dependent on subgroup A virus while secondary events require the presence of the subgroup B virus.

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