**“Stem Cell” Origin of the Hematopoietic Defect in Dyskeratosis Congenita**

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We have used the long-term bone marrow culture (LTBMC) system to analyze hematopoiesis in three patients with dyskeratosis congenita (DC), two of whom had aplastic anemia, and the third had a normal blood count (apart from mild macrocytosis) and normal BM cellularity. Hematopoiesis was severely defective in all three patients, as measured by a low incidence of colony-forming cells and a low level of hematopoiesis in LTBMC. The function of the marrow stroma was normal in its ability to support the growth of hematopoietic progenitors from normal marrows seeded onto them in all three cases, but the generation of hematopoietic progenitors from patient marrow cells inoculated onto normal stromas was reduced, thus suggesting the defect to be of stem cell origin. The parents and unaffected brother of one of the families have also been studied in LTBMC and all showed normal hematopoietic and stromal cell function. From this study we speculate that there are some similarities between DC and the defect in the W/W" mouse.

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**DYSKERATOSIS** congenita (DC) is a rare congenital and familial disorder. The major clinical manifestations are abnormal skin pigmentation, nail dystrophy, and leukoplakia of mucosal membranes. Other features less commonly reported include telangiectasiae, hyperhydrosis, hyperkeratosis or atrophy of palms and soles, defective bones and teeth, sparse fine hair, gastrointestinal hemorrhage, intracranial calcification, ocular abnormalities, microcephaly, and mental and growth retardation. Hitherto, leukemia has not been reported in any case; however, two cases of acute myeloid leukemia (AML) in DC have been documented by our group, with one case being the brother of patient 3 (I. Dokal et al, unpublished observation).

Most cases show X-linked inheritance, but both autosomal dominant and autosomal recessive cases have also been documented. A locus for the DC gene has been mapped to chromosome Xq28 in a family in which DC was X-linked by some analysis in DC is reported as normal in most cases, although an increase in sister chromatid exchange has been documented. More recently, De Bauche et al8 have shown increased susceptibility to chromatid breakages induced by X irradiation in DC.

Studies of the pathogenesis of the BM failure in DC have been limited to clonogenic assays. A marked reduction or absence of multilineage colonies (colony-forming unit-mixed [CFU-Mix]), early and late erythroid colonies (burst-forming unit-erythroid [BFU-E] and CFU-E), and granulocyte-macrophage colonies (CFU-granulocyte-macrophage [CFU-GM]) have been consistently reported. The absence of a serum inhibitor of CFU-GM was documented in one case,13 and the absence of T-cell-mediated hematopoietic suppression in a further case. Although Hanada et al12 reported one case of T-cell-mediated suppression of CFU-GM (but not of CFU-E), this effect was not seen after later splenectomy or recurrence of the pancytopenia. To study the hematopoietic defect in DC in more depth, we have analyzed hematopoiesis in three patients with DC using long-term BM culture (LTBMC)14,15 and a modification of this system to assess separately hematopoietic (“stem cell”) and stromal cell function.16

**MATERIALS AND METHODS**

**BM Samples**

BM was collected into an equal volume of 15% fetal calf serum (FCS) in Iscove’s modified Dulbecco’s medium (IMDM; GIBCO, Grand Island, NY) or RPMI-1640 medium (GIBCO) containing preservative-free heparin (125 U/mL). Samples were taken simultaneously with those for diagnostic purpose or routine review. Informed written consent was obtained from patients 1 and 3 and the parents of patient 2, according to the practice of the local Hospital Ethical Committee. BM samples were also obtained from normal BM transplant (BMT) donors at the time of their marrow harvests, after informed consent. Ribs excised at the time of cardiothoracic surgery from patients with no evidence of hemologic disease were also used as a source of normal BM.

**Cell Separation**

BM mononuclear cells were collected by density gradient centrifugation using Picoll-Hypaque (1.077 g/mL; Flow Laboratories, Irvine, UK) at 400g for 30 minutes. The light-density cells at the interface were collected and washed twice in 5% FCS in IMDM before use in clonogenic cultures, and for isolation of CD34+ cells.
Nucleated marrow cells were separated by sedimentation using 1% methyl cellulose to give a final concentration of 0.1%, allowing the red blood cells (RBCs) 30 to 45 minutes to sediment. The marrow cells in suspension were recovered and washed twice in Nucleated marrow cells were separated by sedimentation using 1% red blood cells (RBG) 30 to 45 minutes to sediment. The marrow were isolated as described previously. Briefly, marrow mononuclear cells were incubated with a 1/10 dilution of MY10 (Becton Dickinson, Oxford, UK; HPCA-1) and fluorescein-conjugated rabbit antiserum IgG (F313; Dako Ltd, High Wycombe, UK) at a dilution of 1/15, and the cells sorted using the fluorescence-activated cell sorter (FACS IV; Becton Dickinson). The machine was set for light scatter and the window set to collect cells with low dilution of 1/15, and the cells sorted using the fluorescence-scatter (FLS), comprising blasts and all cells with 'lymphocyte' characteristics but excluding granulocytes, monocytes, and mature and nucleated RBCs. The cells within this window were further analyzed to collect those of high fluorescence (CD34+ windows).

Clonogenic Cultures

A modification of the method of Fauser and Messner was used to analyze CFU-Mix, CFU-GM, and early erythroid progenitors (BFU-E). Mononuclear cells were plated at 10^6 cells/mL and CD34+ cells at 2.5 x 10^5/mL in 1.3% methyl cellulose in IMDM with 30% pretested FCS, 10% 5637 conditioned medium (CM), 1% bovine serum albumin, and 2 U of partially purified human urinary erythropoietin (Terry Fox Laboratories, Vancouver, British Columbia, Canada). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 5% O_2 in air. Aggregates of greater than 50 cells were scored as colonies. CFU-Mix were identified by the presence of both granulocytic and erythroid elements in single colonies. BFU-E consisting of three or more clusters were recognized by their characteristic brick red appearance. Assays were set up in duplicate and colonies scored on day 14.

For monitoring the generation of CFU-GM in the nonadherent layer in LTBM (see below), the CFU-GM assay was used by adding cells to a concentration of 10^5/mL in IMDM to 15% FCS, 20% 5637CM in 0.33% agar. Cultures set up in triplicate were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air, and colonies with greater than 50 cells were counted on days 9 to 11.

Fibroblast colonies (CFU-F) were assayed based on a modification of the method described by Castro-Malaspina et al. BM cells (5 x 10^6) were inoculated in triplicate into 25-cm^2 Falcon (F. Baker Sci, Runcorn, UK) tissue culture flasks in 5 mL of 5% FCS in IMDM. Flasks were gassed with 5% CO_2 in air and incubated at 37°C. On days 3 to 4, the medium was discarded and replaced with fresh medium, and the cultures ended on day 11. Flasks were washed twice with phosphate-buffered saline (PBS), and colonies were fixed with methanol and stained with 1% brilliant crystal violet. Colonies containing greater than 50 cells were scored as CPU-F.

LTBM

Standard LTBM. As described previously, BM cells were inoculated at a concentration of 2 x 10^6/mL into 10 mL of medium comprising IMDM (350 mOsm/Kg), 10% preselected FCS, 10% preselected horse serum, and hydrocortisone at a final concentration of 5 x 10^-7 mol/L. Flasks were gassed with 5% CO_2 in air, incubated at 37°C, and fed weekly by removing one-half of the medium and replacing it with an equal volume of fresh medium. Every week the harvested cells in the nonadherent layer were counted and assayed for CFU-GM in agar. Flasks were also examined weekly for stroma formation and the appearance of hematopoietic foci.

Assay of stromal function. Stromas grown in standard LTBM were irradiated when confluent (3 to 6 weeks) with one dose of 15 Gy. The supernatant was discarded and the culture recharged with marrow cells in 10 mL of fresh LTBM medium. To assess patient stromal function, normal marrow cells were inoculated onto confluent irradiated patient stroma. To assess patient hematopoietic cell ("stem cell") function, patient marrow cells were inoculated onto confluent irradiated normal stroma. For experiments performed in 1986-1987, marrow mononuclear cells (either 10^6 or 5 x 10^6) were inoculated onto irradiated stromas. For later experiments (1989-90), either adherent cell-depleted (ACD) marrow cells or purified CD34+ marrow cells were used instead to reexamine irradiated stromas. The appropriate normal controls were established by inoculating either (1) normal marrow mononuclear cells, (2) normal ACD cells, or (3) normal marrow cells enriched for hematopoietic progenitors expressing the CD34 antigen onto irradiated normal stromas. ACD cell suspensions were obtained by incubating marrow cells in medium plus 20% FCS at a concentration of 2 x 10^6 cells/mL for 1 hour at 37°C, in a plastic flask, a procedure repeated twice. After each incubation, the nonadherent cells were transferred to a new flask and counted at the end of the procedure.

For patient 1 in 1989, insufficient marrow cells were obtained for ACD. Instead, cells harvested from the nonadherent layer of the patient's standard LTBM at week 1 were inoculated onto an irradiated normal stroma. As a control, the same number of cells from a normal LTBM (established during the same week as the patient's LTBM) at week 1, were inoculated onto an identical normal irradiated stroma. The unavailability of a third identical normal stroma precluded the same experiment for patient 2.

The efficiency of adherent cell depletion as a method for removing the majority of stroma forming cells was assessed by inoculating ACD marrow cells into LTBM flasks and monitoring weekly for stroma formation, as reported previously. Furthermore, we have recently shown that normal CD34+ marrow cells do not give rise to stroma formation in LTBM.

Statistical Analyses

Before analysis, the cell count and CFU-GM data from LTBM were subjected to a logarithmic transformation of the form Y = ln (Y + 1). Means, standard errors, and confidence intervals were calculated using the transformed data. The data for cell counts and CFU-GM were normalized by the number of cells seeded, and the zero time point excluded from the analysis. Standard two-way analysis of variance was used to determine the significance of differences between single cultures versus a control group.

CLINICAL AND CYTOGENETIC DATA

A summary of the major clinical features of the three patients studied is shown in Table 1. All patients showed the classic signs of DC, namely reticular pigmentation, leukoplakia, and nail dystrophy. Also included in Table 1 are the cytogenetic data from analyses of peripheral blood lymphocytes, BM, and skin fibroblasts.

RESULTS

Clonogenic Cultures

Results of clonogenic cultures of BM are shown with corresponding peripheral blood counts in Table 2. When
first examined in 1986, patients 1 and 2 had low normal CFU-GM and normal BFU-E. Three years later, colony numbers had decreased dramatically in both patients, even though patient 2 continued to have less severe aplastic anemia (AA) than patient 1. Fibroblast colonies in both patients were at the upper end of the normal range and later increased in patient 2, showing very florid cell growth and large colony size compared with controls. One brother who has been studied, although without clinical evidence of DC or AA, had low normal CFU-GM and BFU-E, and a CFU-F growth pattern with complete confluency of the flasks by day 11 of culture. In addition, the father had low normal CFU-GM and CFU-F at the upper limit of normal. Patient 3 with DC, who had almost normal hematologic parameters (but with mild macrocytosis), exhibited normal CFU-Mix numbers but CFU-GM, BFU-E, and CFU-F levels were all at the lower limit of normal.

LTBMC

A confluent, microscopically normal stroma was formed in LTBMC by patients 2 and 3 and the parents and brother of patients 1 and 2. Patient 2 developed a confluent stroma but patient 1 developed a partially confluent (60%) stroma, and in both cases very few fat cells were seen. The number of hematopoietic foci was estimated to be less than 10% for patients 1 and 2 and 25% for patient 3, compared with normal LTBMC. Severe functional hematopoietic defects were observed in patients 1 and 2 compared with normal controls (Fig 1A and B), which were more marked for granulocyte-macrophage colony-forming cells (GM-CFC) than for total cell numbers. The LTBMC of patient 3 could only be monitored up to week 3 (showing suboptimal hematopoiesis) because the LTBMC stroma was then used to assess stromal function. The LTBMC established from the parents and brother of patients 1 and 2 (Fig 1A) generated total nucleated cell counts that were not subnormal (CFU-GM generation was not assessed).

Seeding of Hematopoietic Cells on Irradiated BM Stroma

Separate assessment of stromal and "stem cell" function was performed using crossover LTBMC (see Materials and Methods).

Table 1. Clinical and Cytogenetic Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Age at Diagnosis (yr)</th>
<th>Main Clinical Features</th>
<th>Family History (mode of inheritance)</th>
<th>Karyotype</th>
<th>DEB/MMC Test on PBL</th>
<th>Skin Fibroblasts</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20/F</td>
<td>15</td>
<td>Acute retinal vasculitis. Required regular oxymethalone for aplasia.</td>
<td>These patients are sisters. Two brothers are unaffected. (autosomal recessive)</td>
<td>46XX</td>
<td>Normal</td>
<td>—</td>
<td>Died age 20 yr, sepsis/age anaemia</td>
</tr>
<tr>
<td>2</td>
<td>12/F</td>
<td>7</td>
<td>Asymptomatic. Mild aplasia, no treatment.</td>
<td>—</td>
<td>46XX</td>
<td>Normal</td>
<td>—</td>
<td>Remains asymptomatic</td>
</tr>
<tr>
<td>3</td>
<td>25/M</td>
<td>23</td>
<td>Asymptomatic. FBC and BM normal.</td>
<td>His only brother had DC and AML. One nephew has DC. (X-linked recessive)</td>
<td>46XY, inv 8 in all metaphases</td>
<td>Normal (a) inv 8 in all metaphases</td>
<td>Remains asymptomatic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: DEB/MMC test, diethylpropionate and mitomycin C stress test; PBL, peripheral blood lymphocytes; inv 8, pericentric inversion of chromosome 8; t(1;11), der(11) t(1;11); AML, acute myeloid leukemia of French-American-British (FAB) type M5, associated with trisomy 18; FBC, full blood count.

Table 2. Hematologic Data and Clonogenic Marrow Cultures Performed at Time of LTBMC Study

<table>
<thead>
<tr>
<th>Year</th>
<th>Patient</th>
<th>Hb (g/dL)</th>
<th>MCV (fL)</th>
<th>Retic (%)</th>
<th>WBC (×10^3/L)</th>
<th>Neut (×10^3/L)</th>
<th>Plt (×10^3/L)</th>
<th>CFU-Mix</th>
<th>CFU-GM</th>
<th>BFU-E</th>
<th>CFU-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986/87</td>
<td>1</td>
<td>14.4</td>
<td>99</td>
<td>1.0</td>
<td>3.0</td>
<td>0.8</td>
<td>68</td>
<td>27 ± 0.8</td>
<td>66 ± 12.3</td>
<td>56 ± 2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.8</td>
<td>94</td>
<td>2.0</td>
<td>5.9</td>
<td>4.1</td>
<td>128</td>
<td>28 ± 1.5</td>
<td>42 ± 8.8</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>13.2</td>
<td>87</td>
<td>&lt;1.0</td>
<td>7.3</td>
<td>3.7</td>
<td>342</td>
<td>19 ± 5.5</td>
<td>128 ± 5.5</td>
<td>76 ± 7.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>12.4</td>
<td>91</td>
<td>1.0</td>
<td>7.3</td>
<td>4.1</td>
<td>262</td>
<td>50 ± 7.5</td>
<td>71 ± 2.6</td>
<td>39 ± 2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brother</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 ± 8.5</td>
<td>16 ± 5.7</td>
<td>Confluent</td>
<td></td>
</tr>
<tr>
<td>1989/90</td>
<td>1</td>
<td>6.2</td>
<td>106</td>
<td>2.2</td>
<td>1.0</td>
<td>0.4</td>
<td>96</td>
<td>12 ± 4.2</td>
<td>0</td>
<td>56 ± 8.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.4</td>
<td>99</td>
<td>2.0</td>
<td>3.6</td>
<td>2.3</td>
<td>78</td>
<td>1 ± 1.4</td>
<td>1 ± 1.4</td>
<td>103 ± 11.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15.9</td>
<td>97</td>
<td>—</td>
<td>7.5</td>
<td>3.4</td>
<td>229</td>
<td>1.5 ± 0.5</td>
<td>28 ± 2.0</td>
<td>10 ± 1.0</td>
<td>4.3 ± 1.3</td>
</tr>
</tbody>
</table>

Normal controls

Mean ± 1SE

|        | 1.1 ± 0.2 | 41 ± 4.6 | 22.7 ± 4.8 | 27.3 ± 4.2 |
| Range  | 0.3-3     | 8-125    | 3-73       | 3-77       |

n: 21 40 21 24

Father, mother, and brother refer to family of patients 1 and 2. CFU-Mix, CFU-GM, and BFU-E plated at 10^5 cells/ml and are expressed as colonies per 10^5 cells; CFU-F grown from 5 × 10^6 BM cells inoculated per flask.

Abbreviations: Hb, hemoglobin; MCV, mean RBC volume; Retic, reticulocytes; WBC, white cell count; Neut, neutrophils; Plt, platelets.

*Blood count on brother reported as normal but data missing.
STEM CELL DEFECT IN DYSKERATOSIS CONGENITA

Assessment of hematopoietic cell ("stem cell") function.

"Stem cell" function in patients 1 and 2 in 1986 (Fig 3A) was severely defective in terms of total cell numbers generated after the inoculation of patient marrow mononuclear cells onto normal irradiated stromas, and later confirmed on repeat study in patient 1 in 1989, with an absence of generation of CFU-GM over 3 weeks (Table 3). Patient 3 also showed reduced generation of CFU-GM using his ACD marrow cells as the inoculum (Fig 3B; P < .01), indicating a marked defect in "stem cell" function, despite normal hematologic parameters (except for macrocytosis), including BM aspirate and trephine biopsy.

DISCUSSION

Using a modification of the LTBMC system that allows for the separate assessment of marrow stromal and stem cell function, three patients with DC were shown to have normal stromal function but severe defects of stem cell function. Two of these patients had AA but the third had no hematologic abnormalities (except for macrocytosis) on standard investigations, including BM aspirate and trephine biopsy.

The clinical features of the patients reported here were typical of DC, apart from the retinal necrosis and retinal vasculitis in one patient. A generalized vasculitis with evidence of endothelial cell activation, resulting in late deaths, was seen in the two patients reported by Berthou et al at 2 and 7 years, respectively, post-BMT. This may represent delayed sensitivity of endothelial cells to radiation and/or chemotherapy or a preexisting endothelial cell defect. The retinal vasculitis reported here may also have been due to an underlying endothelial cell defect of the retinal vessels.

The finding of defective "stem cell" function in all three patients may be relevant to the pathogenesis and outcome of patients with DC. Firstly, approximately 50% of patients...
with DC develop AA. It has recently been shown that a "stem cell" defect is an important pathogenetic mechanism in all cases of acquired AA studied in LTBMC, whereas stromal function is almost always normal. Secondly, until recently no case of acute leukemia occurring in DC has been reported. However, an affected brother of patient 3 presented and died shortly after with hypoplastic AML who had DC but almost normal hematologic findings,16 ACy22 Secondly, until recently no case of acute leukemia occurring in DC has been reported. However, an affected brother of patient 3 presented and died shortly after with hypoplastic AML associated with a clonal cytogenetic marker (trisomy 18) in the marrow. The presence of a clonal chromosomal marker in skin fibroblasts of patient 3 (and his brother) perhaps supports the idea that the defect in DC occurs through an early genetic event in embryogenesis. If stromal function is completely normal in DC, one would have to postulate that the mesodermal defect had preferentially affected stem cell development, perhaps due to an abnormal subset of mesodermal cells. Alternatively, the mesodermal defect may have produced a subtle stromal defect that allowed the stroma to retain its ability to support normal hematopoiesis as assessed in vitro. There is in vivo evidence to support the in vitro findings of adequate stromal function in DC. There are reports of five patients with DC who have been transplanted for AA using HLA-identical sibling donors. All showed trilineage engraftment, three with long-term engraftment for 2, 4, and 7 years, respectively.

Table 3. Number of CFU-GM Measured in the Supernatant of LTBMC of Marrow Cells Seeded on Irradiated Normal Stromas for Assessment of "Stem Cell" Function

<table>
<thead>
<tr>
<th>Weeks of Culture</th>
<th>Normal control</th>
<th>Patient 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>906</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>340</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>118</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>0</td>
</tr>
</tbody>
</table>

Stroma was grown from the same normal BM.

Because the marrow stroma remains host-derived after allogeneic BMT,26 these observations strongly suggest that the marrow stroma in these transplanted patients was functionally adequate.

The assessment of hematopoiesis in LTBMC in patient 3, who had DC but almost normal hematologic findings, showed an identically severe "stem cell" defect to patients 1 and 2 with AA. This may indicate that the LTBMC system is sensitive enough to detect a "stem cell" defect before the later development of BM failure at a time when the peripheral blood count (apart from macrocytosis) and BM cellularity is normal, analogous to recovered acquired AA patients with very nearly normal blood counts after antilymphocyte globulin therapy,16 or BMT with autologous hematopoietic reconstitution (J. Marsh, unpublished observations). However, this does not necessarily predict that patient 3 will later develop AA. It may be that all patients with DC have an inherent hematopoietic stem cell defect, but AA is only manifest in 50% and acute leukemia in a much smaller percentage of cases. The reduced life expectancy of patients with DC due to nonhematologic causes of death1 may have led to an underestimate of the potential for development of AA or leukemia. Similar defects in LTBMC are seen as a manifestation of BM damage in several groups of patients treated with chemotherapy. Such defects persist for several years, and coexist with normal blood counts, and in some cases with normal incidence of progenitor cells.

Although stem cell function in these patients was normal as assessed, minor morphologic abnormalities of the LTBMC stromas were noted. In two patients, there was a marked reduction in fat cell formation. Furthermore, they showed florid CFU-F growth, and their unaffected brother showed a high incidence of fibroblast colonies. We have also observed a deficiency or absence of fat cells in LTBMC in a minority of patients with acquired AA in which the stroma appeared disorganized with swollen rather than spindle-shaped fibroblasts that were laid down in a haphazard manner, giving a transformed appearance to the stroma. However, morphologic abnormalities of LTBMC stroma in AA do not correlate well with stromal function.

The source of inoculated normal marrow cells used to repopulate irradiated stromas varied in this study, according to changing methodologies over the last 5 years. Initially, low-density mononuclear cells were used. A reduction in inoculating dose from 5 x 10^6 to 10^6, later followed by the use of ACD cells, reflected a desire to reduce the number of normal cells with the potential to give rise to stromal elements. However, a crucial contribution of the inoculum to the further development of the stroma is not likely to be critical, especially during the first 3 to 4 weeks of the culture, the minimum time required for the full development of a functional stroma. From previous studies, the use of ACD marrow cells did not contribute to significant stroma formation,16 so it is unlikely that stromal function was influenced by cells contributed by the second inoculum seeded on the stromas of patients 1 and 2. This is supported by later experiments in which a highly purified population of hematopoietic progenitors composed of cells expressing...
the CD34 antigen and with low right angle and forward light scatter was used to eliminate any residual stromal elements. Also, the development of hematopoiesis in reinoculated cultures was similar when ACD marrow cells and when CD34+ marrow cells were used in these and in previous experiments performed to analyze "stem cell" function.16,17

Finally, there are striking phenotypic similarities of DC to two genetically anemic strains of mice. These mice show coat color abnormalities, sterility, macrocytic anemia, and mast cell deficiency,30 abnormalities in megakaryopoiesis31 and reduced granulopoiesis.32 The stem cell defect in the W/W mouse is due to mutations of the W locus leading to a defective receptor (c-kit) from which the newly described stem cell factor is a ligand.33-35 In the SI/SI' mutant mouse, the microenvironmental defect responsible for the hematopoietic syndrome is due to decreased production of stem cell factor.33-35 The stem cell defect in the W/W mouse can be corrected in vitro and in vivo by marrow transplantation from an SI/SI' mouse because stem cell function in the latter is normal.36 With the findings of abnormal "stem cell" function and normal stromal function in DC presented here, DC would appear to resemble hematologically and phenotypically the defect in the W/W mouse. Studies of the receptor encoded by c-kit and of the binding characteristics of stem cell factor in these patients may be justified.

ACKNOWLEDGMENT

We thank Andrea Campbell for superb technical assistance.

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"Stem cell" origin of the hematopoietic defect in dyskeratosis congenita

JC Marsh, AJ Will, JM Hows, P Sartori, PJ Darbyshire, PJ Williamson, DG Oscier, TM Dexter and NG Testa