CASE REPORT

Idiopathic pure red cell aplasia: first report on CD8 positive lymphocytosis in bone marrow biopsy sections

K M A Ramadan, J A M Anderson, M F McMullin, G M Markey

There is no information in the literature regarding the lymphocyte content or type in bone marrow biopsies from patients with “idiopathic” pure red cell aplasia (PRCA). This report describes the bone marrow biopsy sections of a patient with PRCA. A diffuse CD3 positive (CD8 positive, granzyme B negative) lymphocytosis of approximately 1500/mm² was revealed by immunohistochemical staining. The extent of the T cell increase was not evident from morphological examination of the bone marrow aspirate or biopsy, from flow cytometric analysis of the aspirate, or from the peripheral blood lymphocyte count. Therefore, immunohistochemical analysis should be performed routinely in this rare disease and the data acquired may help to inform the choice of treatment.

Many causes of pure red cell aplasia (PRCA) have been described—for example, B type lymphoproliferative neoplasia and occasionally T large granular lymphocytic (LGL) leukaemia, thymoma, autoimmune diseases, parvovirus B19 infection, and various drugs—but in 46% of cases no cause is demonstrable. A variety of treatments (prednisolone, cyclosporin, antilymphocyte globulin, plasmapheresis, intravenous immunoglobulins, cyclophosphamide, and azathioprine) appear to be instituted on a fairly ad hoc basis, based on their variable success rates. Despite the differing lymphocyte subset targets of successful treatments, there has been little investigation of the presence and/or the nature of the lymphocyte populations involved in these “idiopathic” cases, and no investigation of the absolute lymphocytic content and its type in bone marrow biopsies from these patients. The only information from idiopathic cases appears to be occasional reports of an increase in an activated CD8 positive lymphocyte subset of non-clonal origin in peripheral blood or bone marrow aspirates. Our case report adds to knowledge of the pathogenesis of this disease and indicates the potential clinical usefulness of examining in detail the content and type of lymphocytes in bone marrow biopsies from patients with this rare disease.

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CASE REPORT AND RESULTS

A 43 year old man presented with shortness of breath and palpitation for two weeks having lost 5 kg in weight over the previous two months. There was no drug history of note. On examination, he had no lymphadenopathy or organomegaly. His haemoglobin was 42 g/litre, white cell count was 5.1 × 10⁹/litre, lymphocytes were 1.36 × 10⁹/litre, platelets were 497 × 10⁹/litre, mean corpuscular volume was 105 fl, and the reticulocyte count was 2.4 × 10⁹/litre (normal range, 50–200 × 10⁹/litre). Autoimmune screen (antinuclear, nucleolar, mitochondrial, smooth muscle, and gastric parietal cell antibody) and cytoplasmic and perinuclear antineutrophil cytoplasmic antibodies were negative.

A direct antiglobulin test was negative and vitamin B12 and folate values were normal. Serum erythropoietin was 7876 mIU/ml (normal range, 2.5–10.5). The erythrocyte sedimentation rate was 19 mm/hour. Iron studies and thyroid function tests were normal. Total bilirubin was 4 μmol/litre (normal range, 3–18) and lactate dehydrogenase was 506 U/litre (normal range, 360–720). Parvovirus IgM, human immunodeficiency virus, hepatitis B virus, and hepatitis C virus screens were negative. Paroxysmal nocturnal haemoglobinuria screening by peripheral blood flow cytometry after labelling with anti-CD55/59 was normal. A computerised tomography scan of the chest did not show a thymic mass. Oesophagogastroduodenoscopy showed a prepyloric ulcer and the patient had a positive CLO test. There was no evidence of coeliac disease in the biopsies.

A morphological examination of the bone marrow aspirate showed the absence of erythroid precursors, with 89% myeloid cells and 11% lymphocytes, none of which had LGL morphology. Flow cytometry revealed that 92% of the lymphocytes were T cells (CD3/4, +28%; CD3/8+, 63%; CD3+/HLADR+, 38%) and 1.5% were B cells. Haematoxylin and eosin and Giemsa stained bone marrow biopsy (1.9 cm in length) sections showed the absence of erythroid precursors, no obvious diffuse increase of lymphocytes (fig 1A), and two lymphocytic aggregates, the largest measuring approximately 0.025 mm². Lymphocytes appeared to be extending from the aggregates into the surrounding bone marrow (fig 1B). Immunocytochemical staining was performed on sections of the patient’s Bouin’s fluid fixed bone marrow trephine biopsy using anti-CD3, anti-CD4, anti-CD8, anti-CD20, anti-granzyme B, and anti-glycoporphin A monoclonal antibodies with avidin–biotin visualisation in an automated immunocytochemical analyser. The diffusely scattered positive cells were counted as described previously.

In brief, the visual field area of the ×25 objective of the light microscope used was 0.26 mm²; the positive cells in four consecutive fields of representative areas were counted. Figure 1C shows the pronounced diffuse increase in CD3+ T cells (CD3, ~1200/mm²; CD4, ~73/mm²; CD8, ~970/mm²; granzyme B+, ~48/mm²). CD 20+ cells were ~11/mm² and very occasional groups of two to three glycoporphin A+ erythroblasts were present. T cell receptor gene rearrangement (β and γ) was not demonstrated using primers and a gene scanning technique described previously.

Abbreviations: LGL, T large granular lymphocytic; PRCA, pure red cell aplasia
PRCA, which is currently “idiopathic.”

that he has a “benign” lymphocytosis responsible for his
the LGL type or granzyme B positive. To date, the evidence is

Anti-CD3 immunostained section.

Figure 1  (A) Haematoxylin and eosin (H&E) stained section of bone
marrow biopsy. (B) Lymphocytic aggregate in H&E stained section. (C)
Anti-CD3 immunostained section.

Our patient was treated with prednisolone at 1 mg/kg/day
with a good response: his haemoglobin rose rapidly to 128 g/
litre. His haemoglobin value is currently maintained on
10 mg of prednisolone daily, although attempts to reduce this
further resulted in a drop in haemoglobin. Peripheral blood
lymphocyte immunophenotyping after the initiation of
prednisolone treatment showed activated T cells. The white
blood cell count was $6.7 \times 10^9$/litre, 14% of which were
lymphocytes. Flow cytometry showed that 90% of his
lymphocytes were CD3+ (CD3+/4+, 47%; CD3/8+, 42%; CD3/
26+, 55%; CD3+HLADR+, 20%). He received triple therapy to
eradicate Helicobacter pylori.

DISCUSSION
The presence of lymphocyte aggregates in our patient’s biopsy
and the apparent extension of lymphocytes from the
lymphoid aggregates into the surrounding bone marrow
raised the possibility of a lymphoproliferative neoplasia
underlying the PRCA. T cell receptor gene rearrangement
studies were negative using a technique that has been
reported to detect rearrangements in 98% of patients with T
cell clonal disease.” In addition, the lymphocytes were not of
the LGL type or granzyme B positive. To date, the evidence is
that he has a “benign” lymphocytosis responsible for his
PRCA, which is currently “idiopathic”.

There is very little histopathological data in the literature
regarding the lymphocytic content of bone marrow biopsies
in idiopathic PRCA. There appears to be only one report of a
nodular and diffuse infiltrate of small lymphocytes in the
bone marrow biopsy of a patient who was, however,
subsequently shown to have T cell receptor gene rearrange-
ment and T cell LGL leukaemia. LGL leukaemia/lymphoma
was excluded in our patient. There are occasional reports
of the occurrence of an increased proportion of an activated
CD8+ T cell subset demonstrated in bone marrow aspirate
samples by flow cytometry, as was the case in our patient.”

However, this gives no indication of the size of this
population—in our patient only 11% of the aspirated cells
were lymphocytes, whereas immunostaining of the biopsy
revealed the presence of a very large T cell population. This
demonstration of the size of the lymphocytic population by
the immunohistochemical evaluation of the bone marrow
biopsy was unexpected. Also striking was the fact that the
peripheral blood lymphocyte count was normal despite the
presence of a high number of T cells in the bone marrow
biopsy. Another instructive feature of this case was that the
magnitude of the lymphocyte increase revealed by immu-
nostaining had not been apparent either on haematoxylin
and eosin or Giemsa stained sections, posing the question of
whether similar sized populations are common in PRCA but
are missed without immunohistochemical staining.

“...the peripheral blood lymphocyte count was normal
despite the presence of a high number of T cells in the
bone marrow biopsy”

Whether there is such a thing as a “normal” level of
polyclonal B and T cells in bone marrow from patients who
have bone marrow examination has not been established, but
we regularly see (unpublished observations) 50–200 CD20+
or 100–300 CD3+ lymphocytes/mm³ in situations where
neither is neoplastic. We can then be confident that in our
patient the T cells were greatly increased. The increase was
predominantly of the granzyme B−, CD8+ subtype, enabling
us to predict a response to steroids/cyclosporin/antilympho-
cyte globulin treatment should spontaneous remission not
occur (as proved to be the case). A response to rituximab in a
patient who failed to respond to cyclosporin has also been
described.” Rituximab treatment would have been inap-
propriate in our patient. However, it is possible that the
examination of lymphocyte subsets in the bone marrow
biopsies of patients with idiopathic PRCA will reveal
instances in which the B cell population is increased and
more likely to be the subset involved. It is also possible that
the size of the CD3+ population is related to responsiveness
to treatment. Although our patient responded rapidly to
prednisolone, he requires a relatively high maintenance dose

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**Take home messages**

- We report a diffuse CD3 positive (CD8 positive, granzyme B negative) lymphocytosis of approximately 1 500/mm³ in a patient with “idiopathic” pure red cell aplasia revealed by immunohistochemical staining of bone marrow biopsy sections.
- The extent of the T cell increase was not evident from morphological examination of the bone marrow aspirate or biopsy, from flow cytometric analysis of the aspirate, or from the peripheral blood lymphocyte count.
- We suggest that immunohistochemical analysis should be performed routinely in this rare disease and that the data acquired may help to inform the choice of treatment.
and may require more specific anti-T cell treatment in the future.

PRCA is a rare disorder. Antibodies that enable a considerable degree of lymphocyte subtyping in bone marrow biopsies are now available and determination of absolute numbers of the various lymphocyte subsets in bone marrow biopsies is a quick and easy process that would permit interlaboratory comparability of data. We suggest that lymphocyte subtyping performed on biopsy sections should be incorporated into the investigation of these patients because there is information, which may inform treatment, yet to be acquired.

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The patient gave full consent for this case report to be published

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