Pure red cell aplasia associated with malignant thymoma, myasthenia gravis, polyclonal large granular lymphocytosis and clonal thymic T cell expansion

S I Handa, K P Schofield, M Sivakumaran, M Short, R S H Pumphrey

Abstract
A case with the triad of pure red cell aplasia (PRCA), myasthenia gravis, and malignant thymoma is reported. There was a clonal proliferation of T cells within the thymoma, as demonstrated by a T cell antigen receptor (TCR) \( \delta \) chain gene rearrangement. However, despite a large granular lymphocytosis, clonality could not be shown in the peripheral blood either before or after thymectomy. There was no evidence of human T cell lymphotrophic virus type 7 (HTLV1) infection.

It is postulated that the clonal thymic T cell population secreted cytokine(s), which stimulated the polyclonal proliferation of large granular lymphocytes, which in turn suppressed erythropoiesis. Thymectomy removed the stimulus to the large granular lymphocytes and hence there was a resurgence of erythropoiesis.

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Pure red cell aplasia (PRCA) is characterised by normocytic anaemia, reticulocytopenia, and severe erythroid hypoplasia of the bone marrow associated with normal myeloid and megakaryocytic cell lines. It is divided into congenital and acquired categories, the latter being further subdivided into two sub-classes—primary and secondary. About 50% of patients with PRCA have a thymoma. Conversely, because PRCA is rare, it occurs in only 5% of patients with thymoma. There is also a well recognised association between thymomas and myasthenia gravis. However, the simultaneous occurrence of all three disorders is extremely rare. Some cases of so-called primary PRCA have large granular lymphocytic leukaemia. This is a relatively recently recognised condition characterised by a neoplastic proliferation of large granular lymphocytes.

Case report
A 73 year old man presented with a four week history of symptoms of anaemia. His blood count showed a haemoglobin of 41 g/l with a mean corpuscular volume (MCV) of 100 fl, a mild lymphocytosis (5.7 \( \times \) 10\(^9\)/l), and a normal platelet count. Serum vitamin B12 and folate concentrations were normal. A bone marrow aspirate confirmed a diagnosis of pure red cell aplasia. Although a chest radiograph showed changes of chronic airways disease only, a computed tomography scan of the thorax showed a soft tissue mass in the anterior mediastinum that was suspicious of thymoma. There was a polyclonal increase in globulins with raised IgG and IgM. A random blood glucose sample showed an increased concentration and a glucose tolerance test was consistent with diabetes mellitus. He was initially managed with blood transfusions.

A month after presentation, he developed a unilateral partial ptosis. He also admitted to intermittent diplopia, some leg weakness, and non-progressive dysphagia. On examination he had a left partial ptosis and bilateral shoulder abduction weakness on exercise. A ten-silon test was positive. Antibodies to acetylcholine receptor were increased and he later developed antibodies to striated muscle. Myasthenia gravis was diagnosed and he was given Pyridostigmine.

While awaiting thymectomy, his peripheral blood lymphocyte count increased, reaching a peak of 12.1 \( \times \) 10\(^9\)/l of which about 30% were large granular lymphocytes. Immunopheno-typing of the peripheral blood and bone marrow lymphocytes showed that about 70% were T cells (67% with CD8 and 2% with CD4 T cell antigen receptors). About 30% of the peripheral blood mononuclear cells were of the natural killer cell phenotype (CD2+, CD3-, CD7+, CD16+, CD57+). A monoclonal antibody panel containing seven antibodies against the variable regions of the TCR showed the peripheral blood T lymphocytes to be polyclonal. Infection with HTLV
1 was excluded by serological, western blotting and molecular studies.

Thymectomy was performed and a large (13 × 9 × 7 cm) lobulated tumour that had invaded the pericardium was resected. Histological examination showed a thymoma of mixed epithelial—lymphoid type which was confirmed by immunohistochemistry. In view of incomplete encapsulation and extension into mediastinal fat, it was regarded as malignant. Immunophenotyping of the lymphoid cells from the thymoma showed a normal thymus pattern (CD3 = 30%, CD4 = 82%, CD8 = 77%, CD1 = 77%, CD2 = 97%, CD5 = 27%, CD7 = 95%, CD38 = 95%, CD20 = 1%, CD23 = 1%, CD34 = 1% and CD25 = 2%). T cell receptor gene rearrangement studies on the thymoma tissue revealed a clone with rearrangement of the δ gene (figure).

Within a few hours of surgery the peripheral blood lymphocyte count fell to within the normal range and remained there. The large granular lymphocytes fell to 12% of the total peripheral blood lymphocytes. There was a corresponding change in the bone marrow lymphocyte surface markers. Molecular studies on the peripheral blood lymphocytes using TCRβ chain constant region and γ and δ chain joining region probes showed no evidence of monoclonal proliferation of T lymphocytes.

A bone marrow aspirate four weeks after surgery showed a resurgence of erythropoiesis and he became transfusion independent. He died about six months after initial presentation following a protracted stay in intensive care with lower respiratory tract infection. Though a necropsy was not carried out, cause of death was thought to be severe bronchopneumonia.

Methods

Initial screening tests for HTLV1 used both the Abbott enzyme immunoassay (EIA) (Abbott Diagnostics, Maidenhead, Berks) and the Fuji Rebio Serodia HTLV1 particle agglutination test (Mast Laboratories Limited, Bootle, Merseyside). The HTLV1 western blot used was from Du Pont (UK) Limited, Stevenage, Herts. All tests were carried out following the manufacturers’ protocols.

The presence of HTLV1 sequences was looked for by extracting DNA from the thymoma tissue by standard procedures using sodium dodecyl sulphate/Proteinase K digestion. The DNA was digested with EcoRI or SacI, separated on a 0.8% agarose gel, and transferred to GeneScreen membranes. These blots were probed with an 8–9 kilobase SacI fragment of the pMT2 clone which had been labelled with 32P by the random priming method, and washed under conditions of high stringency. The blot was autoradiographed.

DNA from peripheral blood mononuclear cell preparations and thymic tissue was extracted using the phenol-chloroform method. DNA (10 μg) was digested with BamHI and Hind III restriction enzymes (and additionally with EcoRI when these results were inconclusive) and the DNA fragments separated by 0.8% agarose gel electrophoresis. These were transferred to nitrocellulose membranes by vacuum blotting, ultraviolet light fixed, and hybridised with the following 32P-labelled probes: the constant TCRβ region (Cβ), M13B110B1; the TCRγ joining region, pPH60; and the TCRδ joining region, R21XH. Hybridised filters were washed and the radioactive signal monitored by autoradiography.

A suspension of thymic cells was prepared by macerating the thymic tissue. Purified thymic and bone marrow mononuclear cell suspensions were obtained by density gradient centrifugation with lymphocyte separation medium (ICN, Irvine). Peripheral blood samples were stained without separating the cells.

A monoclonal antibody panel was selected to identify specific differentiation antigens. T cell receptor variable regions were studied using the Diversi-T TCR screening panel 1A (T cell Sciences, Cambridge, Massachusetts).

The cells were incubated with optimal concentrations of each monoclonal antibody (manufacturer’s recommendations) for 30 minutes at 4°C. The cells were then washed twice in Hanks’ Balanced Salt Solution (Life Technologies, Paisley, Scotland) containing 0.1% sodium azide, and incubated for a further 30 minutes at 4°C with 10 μl fluorescein-isothiocyanate conjugated affinity purified goat anti-mouse immunoglobulin (Becton Dickinson, Oxford). The cells were washed twice as before. The red cells in the whole blood samples were lysed and the leucocytes fixed using the Coulter Q—prep system (Coulter Electronics Limited, Luton, Beds), whereas the purified mononuclear cells were fixed using 1% paraformaldehyde.

Flow cytometric analysis was performed using a Coulter EPICS Profile II flow cytometer.
Discussion
Pure red cell aplasia has been described in conjunction with numerous conditions, including immunological disorders (such as thymoma, haemolytic anaemia, connective tissue disorders), lymphoproliferative and stem cell disorders, pregnancy, nutritional deficiencies and thyroid carcinoma. On the other hand, neoplasms of the thymus are associated with more than 20 "parathymic" syndromes affecting about 40% of patients.

The three most common associations are with myasthenia gravis, PRCA, and hypogammaglobulinaemia. A review of the published studies revealed 22 patients with the clinical triad of PRCA, myasthenia, and thymoma, and of these, in only 14 cases was thymic pathology documented. Starkebaum et al first provided preliminary evidence suggesting that large granular lymphocytic leukaemia may be associated with infection with a retrovirus related to HTLV. Subsequently, Levitt et al provided very convincing evidence of HTLV1 infection in a patient with large granular lymphocytic leukaemia, raising the possibility of an aetiological link between them. More relevant to our case, however, is the finding that Oon et al had earlier shown retrovirus-like particles in all cases of human thymomas and thymus hyperplasias examined by electron microscopy. They also provided additional convincing evidence of retroviral infection in these patients and suggested that this may be involved in the genesis of the thymic disorders.

Several humoral as well as cell mediated mechanisms have been demonstrated in both primary and secondary PRCA except in B CLL where T cell suppression primarily accounts for the red cell aplasia. A case has been described in which it was shown that only the cell adherent layer of the bone marrow, containing mostly macrophages, was implicated in the pathogenesis of the PRCA. In large granular lymphocytic leukaemia a direct inhibitory effect of abnormal large granular lymphocytes on erythropoiesis in vitro has been shown in some patients with associated PRCA.

Several investigators have recently shown that a significant proportion of patients with large granular lymphocytosis have clonal proliferations, evidenced by rearrangement of the TCR genes. Occasionally, rearrangement of the TCR γ gene with a germline TCR β gene has been found. Our patient had a clonal proliferation of T cells within the thymoma, as evidenced by the finding of a rearranged TCR δ gene. However, no clonal proliferation of T cells could be shown in the peripheral blood either before thymectomy (by immunological methods) or after thymectomy (by molecular methods).

Thymomas of mixed epithelial-lymphoid type are epithelial in nature and the lymphoid element is usually reactive or non-neoplastic. Having demonstrated the presence of a clonal lymphoid population in this patient's thymoma, we question whether the lymphoid component of the thymoma was also neoplastic or whether there were two distinct neoplasias—namely, epithelial lymphoma and T cell lymphoma. Occurrence of both T cell and B cell lymphomas within the thymus gland is well recognised. A normal immunophenotypic profile of thymoma thymocytes does not necessarily exclude T cell lymphoma as it has been shown that the antigen phenotype of thymoma thymocytes is similar not only to that of normal thymocytes but also to that of the neoplastic cells of T cell lymphoblastic lymphoma. Our case clearly shows this possibility despite the presence of a clonal T cell population, the immunophenotype of the thymoma tissue was that of a normal thymus.

There was a precipitous fall in the peripheral blood absolute lymphocyte count after surgery due largely to a decrease in circulating large granular lymphocytes. There was a corresponding fall in the bone marrow, accompanied by a resurgence of erythropoiesis. We postulate the following model to explain these observations. The clonal population of T cells in the thymus may have been secreting cytokine(s) which stimulated the development of large granular lymphocytes which in turn suppressed erythropoiesis. The removal of the source of the cytokine(s) by thymectomy would remove the stimulus to the large granular lymphocytes and hence the inhibition of erythropoiesis. The postulated reactive nature of the peripheral blood large granular lymphocytes would explain why we were unable to demonstrate clonality. Our findings are in contrast to a recent report of clonal T cell populations in the peripheral blood in two out of three cases of PRCA, one of which was associated with B cell chronic lymphocytic leukaemia (B CLL) and the other of which may have had early B CLL.

This case shows that the distinction between primary and secondary PRCA is no longer clear. Until now, patients in whom PRCA is associated with an absolute or relative T lymphocytosis have been included in the primary category. However, this patient had both an associated thymoma (putting it in the secondary category) as well as an absolute T lymphocytosis. We propose, therefore, that the term primary or idiopathic PRCA should be reserved for the small but significant minority of cases in whom no associated T lymphocytosis or other form of suppression can be shown. Cases with an absolute T cell lymphocytosis should therefore be included in the secondary category.

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3 Bailey RO, Dunn RG, Rubin AM, Ritecieo AL.
Concealed homicidal strangulation first discovered at necropsy

D W Sadler

Abstract

One adult and one childhood case of concealed homicide by strangulation are presented. Both cases were first recognised on formal neck dissection at necropsy. Neither showed any obvious external trauma to the neck or laryngeal fracture. External petechiae were absent in the child. The pattern of internal injuries in both cases suggested that the likely mechanism was strangulation. Particular care is required in busy routine medicolegal practice in order to detect deaths which are not immediately apparent as asphyxial.


Case reports

Case 1

The body of a 33 year old man was found lying face down on a country track. Although the first police officer on the scene had been concerned, the consensus view of CID officers and the police surgeon, after examining the body, both at the locus and stripped of clothing at the police mortuary, was of sudden natural death. The case was referred for routine medicolegal necropsy the following day. Occasional minor fresh abrasions were present on the forehead and elbows. There was a 4 × 1 mm laceration to the frenulum of the upper lip. There were florid petechiae over the eyelids and conjunctiva. There were no externally apparent neck injuries. Neck dissection revealed bruising in the left platysma, sternomastoid, omohyoid and sternohyoid muscles, in both inferior pharyngeal constrictor muscles overlying the superior horns of the thyroid cartilage and over the upper right carotid sheath. The thyroid cartilage and hyoid bone were intact. There were florid mucosal haemorrhages, ranging from 1–7 mm, over the lateral and anterior walls of the oropharynx and posterior surface of the epiglottis. On the right edge of the tongue was an area of haemorrhage 8 mm in maximum dimension. Dissection of the back revealed fresh bruising within the back muscles, either side of T11 level and to the left of L1 level. No further trauma or any clinically relevant natural disease were identified at necropsy. Toxicological analyses were negative. The cause of death was given as “strangulation”. After extensive police investigation and a Fatal Accident Inquiry the death is officially viewed as an unsolved homicide.

Case 2

A 6 week old baby girl was found dead, lying face down on a couch after her mother was admitted to hospital for suspected self-poisoning. Suspicion of infanticide, rather than cot death, was raised when the mother recovered completely shortly after hospital admission. The child was well nourished and showed no external trauma. There was an abundant plume of yellow froth at the nostrils. There were no external petechiae. There were occasional petechiae over the visceral pleura, epicardium, and thymus. Neck dissection revealed two discrete areas of soft tissue bruising to the left upper neck, extending deeply around the digastric muscle and adjacent lymph nodes and becoming confluent over the carotid sheath and precervical fascia. Closer examination of the overlying skin revealed a barely discernible 6 mm linear abrasion which became more obvious with drying over the next 24 hours. Dissection of the back of the neck revealed a 17 × 13 mm horizontally aligned bruise straddling the midline within the upper trapezius muscle, 18 mm below the external occipital protuberance. No further trauma or natural disease was identified.

Microbiological screening and radiological...
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