Case report

Large granular lymphocytosis associated with rheumatoid arthritis

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SUMMARY A 74 year old woman with rheumatoid arthritis, hepatosplenomegaly, neutropenia, and peripheral blood lymphocytosis is described. The lymphocytes had a large granular morphology and expressed a CD3⁺ CD8⁺ Leu7⁺ surface antigen phenotype. They did not have natural killer cell function. Southern analysis of the lymphocyte DNA using two restriction enzymes showed a rearranged pattern for the T cell receptor β chain gene, indicating a monoclonal lymphoproliferation. Large granular lymphocytosis is a rare and heterogeneous phenomenon, which has become more clearly characterised through the application of molecular biology techniques. Most cases appear to be forms of T cell leukaemia with a chronic benign course. The association between rheumatoid arthritis and large granular lymphocytosis is emphasised.

Key words: T cell receptor β chain.

Within the last few years a lymphocyte sub-population has been recognised known as the large granular lymphocyte population. Morphological characteristics include a large amount of cytoplasm and azurophilic granules. Most natural killer (NK) cells are found within this population,¹ though it is not homogeneous and includes more than one cell type as defined by monoclonal antibodies to surface antigens.² Abnormal expansion of blood large granular lymphocytes is an uncommon phenomenon with a variety of clinical associations. A syndrome comprising large granular lymphocytosis, neutropenia, and splenomegaly has been well defined.^{3,4} We describe here a case of rheumatoid arthritis associated with a monoclonal expansion of the large granular lymphocyte subpopulation.

Case report

A 74 year old woman presented with a nine month history of peripheral symmetrical polyarthralgia and

morning stiffness. She had a seropositive erosive arthropathy with hepatomegaly of 4 cm and splenomegaly of 3 cm. There was no lymphadenopathy either clinically or on whole body computed tomography scan. Routine biochemical and radiological investigations were normal.

Haemoglobin was 101 g/l, red blood cells $4.3 \times 10^{12}/l$ (normal $3.9-5.6 \times 10^{12}/l$), platelets $298 \times 10^9/l$ (normal $150-400 \times 10^9/l$), white cell count $6.9 \times 10^9/l$ (normal $4-11 \times 10^9/l$), neutrophils $0.47 \times 10^9/l$ (normal $1.5-4.0 \times 10^9/l$), and lymphocytes $6.02 \times 10^9/l$ (normal $1.5-4.0 \times 10^9/l$). Neutrophil antibodies were not detected.

Bone marrow examination showed a marked infiltration with mature looking lymphocytes. Erythropoiesis was micronormoblastic and granulopoiesis was hypoplastic.

Lymphocyte analysis with a panel of monoclonal antibodies was done by indirect immunofluorescence and flow cytometry with a fluorescent activated cell sorter (Becton Dickinson). Natural killer cell function was assessed in a ⁵¹Cr release assay using the K562 human myeloid cell line as target.⁵

The immunophenotyping gave the following results: CD2 (T11) 10% positive, CD5 (T1) 5%,

CD3 (T3) 94%, CD4 (T4) 8%, CD8 (T8) 87%, Leu 7 63%, CD16 (Leu 11) 3%, HLA-DR 44%. About 4% of the cells were B cells (CD24 and surface immunoglobulin positive). In the NK cell functional assay the lymphocytes showed very low cytotoxic activity (<1 lytic unit per 10^6 lymphocytes, normal range 6–80). Most of the cells therefore expressed the abnormal T cell phenotype $CD2^- CD5^- CD3^+ CD4^- CD8^+ Leu\ 7^+ HLA-DR^+$. Neither the NK cell marker CD16 (Leu 11) nor NK cytotoxic function were present.

A Southern blot analysis was carried out for rearrangements of the gene for the β chain of the T cell antigen receptor on DNA extracted from blood mononuclear cells.⁶ A probe to the $C\beta 1$ constant region of this gene was prepared from the

Bgl II fragment of the cDNA Jur- $\beta 2$ clone.⁷ In germ line DNA (non-rearranged) this probe detects fragments of molecular weight 24 kb after digestion with the enzyme Bam HI, and 4 kb and 12 kb using the enzyme Eco RI. Figure 1 shows the results and indicates a rearrangement in the T cell receptor β chain gene, involving the $C\beta 2$ region of one allele appearing as an additional band on the Bam HI digest. The Eco RI digest showed deletion of the 12 kb $C\beta 1$ fragment, leaving only a weak germ line band. These results indicate a monoclonal origin for the cells and are consistent with the abnormal T cell immunophenotype.

Discussion

We have described a 74 year old woman with rheumatoid arthritis of recent onset, hepatosplenomegaly, neutropenia, and large granular lymphocytosis (LGL). This form of lymphocytosis is uncommon. Neutropenia and splenomegaly appear to be almost constant features, and rheumatoid arthritis (RA) is a frequently associated condition. Barton *et al* reviewed 14 such cases,⁴ and Newland *et al* described RA in seven of 21 patients with LGL.³ The condition is distinguishable from Felty's syndrome by the lymphocytosis, although, in both, antineutrophil antibodies and neutrophil binding to immune complexes may play a part in producing neutropenia.^{8,9}

Surface marker and functional analyses have shown LGL to be heterogeneous. Most cases express the surface antigens CD3, CD8, and Leu 7. They therefore appear to represent an expanded T cell subset, though they generally lack or express weakly the 'pan-T' marker CD5 (T1).^{3,10} The case described here was of this phenotype but was unusual in its lack of expression of CD2 (T11).

Although NK cell function is associated among normal blood lymphocytes with the large granular cell,¹ the abnormal lymphocytes of patients with LGL rarely show this activity,^{4,11,12} and their surface markers are those of T cells. Rare cases of LGL with NK function have been described, but they have a distinct immunophenotype, being $CD2^+ (T11)^+$ and $CD16 (Leu\ 11)^+$ without T cell restricted surface antigens (CD3, CD4, CD8) or evidence of T cell antigen receptor gene rearrangement.^{13,14} They therefore represent proliferations of lymphocytes whose phenotype corresponds to normal NK cells.^{15,16}

Most patients with LGL have a benign clinical course, and some are asymptomatic for long periods of follow up.³ The most serious complication of the disease is bacterial infection associated with severe neutropenia. The question of whether LGL is a T cell leukaemia or some form of reactive lympho-

Fig. 1 Southern blot analysis of the T cell receptor β chain gene using the Jur- $\beta 2$ constant region cDNA probe. The partial map of the β chain gene in germ line configuration shows the joining (J) and constant (C) regions, the sites of cleavage by the restriction endonucleases Bam HI (B) and Eco RI (R) and the $C\beta 1$ and $C\beta 2$ regions recognised by the probe. Lanes 1–3 are results of Eco RI digestion of DNA: 1 is from placenta, with germ line bands of molecular weight 4 kb and 12 kb; 2 is a case of T cell acute lymphoblastic leukaemia (T-ALL), with a rearranged band at 6 kb; 3, from blood lymphocytes of the patient described, shows reduced intensity of the 12 kb band. Lanes 4–6 are results of Bam HI digestion: 4, from placenta, illustrates the single 24 kb germ line band; 5, T-ALL, shows two bands indicating rearrangement of both alleles of the T cell receptor β chain gene; 6, DNA from the patient's lymphocytes, shows a 15 kb rearranged band and a weak 24 kb band. These results indicate homogeneous (i.e., monoclonal) rearrangement in the β chain gene, involving the $C\beta 2$ region of one allele, appearing as an additional (15 kb) band on the Bam HI digest.

cytosis was unresolved until the recent application of gene probe analysis allowed determination of the monoclonality or polyclonality of the cells. Such analyses have shown a monoclonal pattern of T cell receptor β chain rearrangement in most cases of LGL with neutropenia.^{6 9 10 17} A few proliferations are polyclonal, and it is not clear whether the LGL associated with RA is always monoclonal. Our patient showed monoclonality, and this lymphocytosis can therefore be regarded as a form of T cell chronic leukaemia. Its relative malignant or benign nature can only be determined by long term follow up.

The association between RA and LGL is interesting, though an aetiopathogenic link between the conditions is not clear. In most reported cases the features of LGL developed only after erosive arthritis had been present for many years. In only two of 14 cases reviewed did Barton *et al* find simultaneous onset of the two conditions.⁴ In most cases, therefore, the T cell lymphoproliferation may be interpreted as a consequence of prolonged abnormal activation of the immune system associated with autoimmunity. A recent report of 12 cases of LGL indicated the presence of antibodies reacting with the retrovirus HTLV-I in six of the patients. Four of these cases had arthritis.⁹ These findings raise the interesting possibility that both the arthritis and the (leukaemic) T cell proliferation may be the result of infection by a T lymphotropic virus related to HTLV-I.

In conclusion, we describe here an unusual case of late onset RA with large granular lymphocytosis and neutropenia. Immunophenotyping and gene probe analysis indicated a monoclonal T cell lymphoproliferation. We draw attention to the clinical association between this type of T cell leukaemia and RA.

Addendum

Since writing this report the patient was admitted as an emergency with peritonitis due to a left pericolic abscess. She died shortly after surgery and at necropsy the immediate cause of death was determined to be coronary insufficiency.

The gene arrangement and immunophenotyping analyses were supported by a grant from the Cancer Research Campaign.

References

- 1 Timonen T, Ortaldo J R, Herberman R B. Characteristics of human large granular lymphocytes and relationships to natural killer and K cells. *J Exp Med* 1981; **153**: 569–82.
- 2 Ortaldo J R, Sharrow S O, Timonen T, Herberman R B. Determination of surface antigens on highly purified human NK cells by flow cytometry with monoclonal antibodies. *J Immunol* 1981; **127**: 2401–9.
- 3 Newland A C, Catovsky D, Linch D, *et al*. Chronic T cell lymphocytosis: a review of 21 cases. *Br J Haematol* 1984; **58**: 433–46.
- 4 Barton J C, Prasthofer E F, Egan M L, Heck L W, Koopman W J, Grossi C E. Rheumatoid arthritis associated with expanded populations of granular lymphocytes. *Ann Intern Med* 1986; **104**: 314–23.
- 5 West W H, Cannon G B, Kay H D, Bonnard G D, Herberman R B. Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterisation of effector cells. *J Immunol* 1977; **118**: 355–61.
- 6 Waldmann T A, Davis M M, Bongiovanni K F, Korsmeyer S J. Rearrangements of genes for the antigen receptor on T cells as markers of lineage and clonality in human lymphoid neoplasms. *N Engl J Med* 1985; **313**: 776–83.
- 7 Yoshikai Y, Anatoniu D, Clark S P, *et al*. Sequence and expression of transcripts of the human T cell receptor β -chain genes. *Nature* 1984; **312**: 512–24.
- 8 Bucknall R C, Davis P, Bacon P A, Verrier-Jones J. Neutropenia in rheumatoid arthritis. Studies on possible contributing factors. *Ann Rheum Dis* 1982; **41**: 242–7.
- 9 Starkebaum G, Loughran T P, Kalyanaraman V S, *et al*. Serum reactivity to human T-cell leukaemia/lymphoma virus type 1 proteins in patients with large granular lymphocytic leukaemia. *Lancet* 1987; **i**: 596–9.
- 10 Berliner N, Duby A D, Linch D C, *et al*. T cell receptor gene rearrangements define a monoclonal T cell proliferation in patients with T cell lymphocytosis and cytopenia. *Blood* 1986; **67**: 914–8.
- 11 Bassan R, Introna M, Rambaldi A, *et al*. Large granular lymphocyte/natural killer cell proliferative disease: clinical and laboratory heterogeneity. *Scand J Haematol* 1986; **37**: 91–6.
- 12 Loughran T P, Draves K E, Starkebaum G, Kidd P, Clark E A. Induction of NK activity in large granular lymphocyte leukaemia: activation with anti-CD3 monoclonal antibody and interleukin 2. *Blood* 1987; **69**: 72–8.
- 13 Tagawa S, Tokymine Y, Ueda E, *et al*. Leu 11⁺ T γ cell chronic lymphocytic leukaemia with partially activated natural killer function and its further activation by recombinant IL2 in vitro. *Blood* 1986; **68**: 846–52.
- 14 Lauria F, Foa R, Migone N, *et al*. Heterogeneity of large granular lymphocyte proliferations: morphological, immunological and molecular analysis in seven patients. *Br J Haematol* 1987; **66**: 187–91.
- 15 Lanier L L, Phillips J H, Hackett J, Tutt M, Kumar V. Natural killer cells: definition of a cell type rather than a function. *J Immunol* 1986; **37**: 2735–9.
- 16 Trinchieri G, Perussia B. Human natural killer cells: biological and pathologic aspects. *Lab Invest* 1984; **50**: 489–513.
- 17 Rambaldi A, Pelicci P-G, Allavena P, *et al*. T cell receptor β chain gene rearrangements in lymphoproliferative disorders of large granular lymphocytes/natural killer cells. *J Exp Med* 1985; **162**: 2156–62.