Two cases of heavy chain disease associated with a different pathology

Umberto Basile[1], Monica Di Nunno[1], Francesca Gulli[1], Giovanni Cigliana[2], Elisabetta Curti[2], Barbara Frollano[2], Giorgio Tasca[3], Mario Sabatelli[3]

[1]Laboratorio di Immunologia e Istituto di Patologia Generale, Università Cattolica del Sacro Cuore, Roma
[2]Laboratorio di Patologia Clinica, Istituto Nazionale dei Tumori "Regina Elena", Roma
[3]Istituto di Neurologia, Università Cattolica del Sacro Cuore, Roma

ABSTRACT

The heavy chain disease (HCD) is a rare B-cell lymphoproliferative disorder that may involve the three main immunoglobulin heavy chain isotypes, i.e. α, γ and μ, whose hallmark is the occurrence in serum of monoclonal heavy chains without associated light chains. HCD may have variable clinical presentations and different histopathologic features. We report two cases of HCD that, unlike most of the previously reported cases, were found in patients affected by a different pathology. The first patient, a 75 years old man, was affected by chronic lymphocytic leukemia and a γ-heavy chain without associated light chain was detected by immunofixation electrophoresis both in patient serum and urine. A monoclonal IgGλ was also detected in serum and a monoclonal free λ chain in urine. The second case refers to a 72 years old man affected by progressive multifocal leukoencephalopathy, showing γ-heavy chain incomplete fragments, both in serum and urine, and severe immunoglobulin deficiency.

INTRODUCTION

The heavy chain disease (HCD) is a rare lymphoproliferative disorder of plasmacells characterized by production of truncated monoclonal immunoglobulin heavy chains without associated light chains. HCDs involve the three main immunoglobulin classes, α-HCD, the most common, γ- and μ-HCD, and have variable clinical presentations and different histopathologic features. The diagnosis of HCD requires the evidence of a deleted immunoglobulin heavy chain without a bound light chain in the serum or urine (1).

Franklin's disease (γ-HCD) is a rare hematological disorder characterized by synthesis of truncated monoclonal γ-heavy chains, mostly dimers with different length, devoid of light chains. γ-HCD has been reported to occur equally in men and women, but in a recently described series there was a clear predominance of women (2, 3). Originally, γ-HCD was considered to be a lymphomatous illness. However, γ-HCD has various clinical and pathologic features (1). In general, γ-HCD can be divided into three categories: 1) disseminated lymphoproliferative disease reported in 57-66% of patients and present in most patients at the time of diagnosis; 2) localized proliferative disease, reported in 25% of patients, in which disease may be extramedullary or may involve only the bone marrow. Cutaneous involvement is the most frequently reported extramedullary presentation; 3) no apparent proliferative disease, reported in 9-17% of patients with γ-HCD. In most of these patients an autoimmune disorder with or without lymphoid proliferation has been reported, including rheumatoid arthritis, autoimmune cytopenias (hemolytic anemia or thrombocytopenic purpura), lupus erythematosus, Sjögren syndrome, myasthenia gravis, thyroiditis and vasculitis (1). Here, we describe two cases of old men affected by chronic lymphocytic leukemia (CLL) and by progressive multifocal leukoencephalopathy (PML), both showing an associated γ-HCD.

CASE REPORT

The first case is a 75 years old man clinically investigated for the first time in March 2006 for symptoms, including fatigue, edema, and severe weight loss. Physical examination identified an unexpected hepatosplenomegaly. A bone marrow biopsy revealed a highly increased cellularity, a low percentage of neutrophil granulocytes (11.0%) [reference interval (RI), 35-65%], and erythrocytes (6%, RI 15-35%) and a highest percentage of lymphocytes (83%, RI 3-15%). Furthermore, a plentiful infiltration of morphologically ripe, small lymphocyte was revealed. In April 2006, the patient's bone marrow was evaluated by a fluorescence in situ hybridization (FISH) analysis by means of LSI p53 probe (17p13.1), a ~145 kb unique sequence probe, LSI D13S319 probe, a ~130 kb unique sequence probe, and LSI ATM (11q22) probe, a ~500 kb unique sequence probe that hybridizes to the 11q22.3 region of chromosome 11 (all probes, labeled with a fluorophore, were from Abbott Molecular), showing the absence of p53 gene, Rb locus (13q14) and ataxia telangiectasia mutated (ATM) gene deletions. Otherwise, the FISH performed using the CEP 12 DNA probe (Abbott Molecular) found 24% of pathological cells showing nuclei with trisomy of chromosome 12. For every probe, the FISH analyzed 200 cells. Four months later, blood cell count showed macrocytic anemia (hemoglobin 110 g/L, hematocrit 33.9%) and leukocytosis (white blood

cells 16.0 x10^9/L). Differential leucocyte count showed an absolute increase of lymphocytes and monocytes, with severe neutropenia. Clinical chemistry testing showed elevated β_2-microglobulin (12.0 mg/L) and lactate dehydrogenase (LDH) (1766 U/L) values, corroborating the diagnosis of CLL. The search for monoclonal proteins (MC) performed by agarose gel immunofixation (IFE) (Hydrasys Sebia) showed the presence in serum of an abnormal broad band of γ-heavy chain not associated with corresponding light chain in β-region and a small MC IgMλ in γ-region (Figure 1). Urine IFE showed the same pattern of unbound γ-heavy chain associated with a monoclonal free λ light chain, i.e. λ Bence Jones proteinuria (Figure 1). Additional investigation was performed by flow cytometry, immunophenotyping the proliferating lymphocytes that typically expressed monotypic surface proteins CD5 (15% of proliferating lymphocytes), CD19 (53%), CD23 (52%), and CD38 (15%), with the evidence of a B-cell lymphoproliferative disorder presenting a λ clone in 15% of the mononuclear cells. In particular, CD38 expression on leukemic lymphocytes is a prognostic factor, adopting a 30% cutoff (4).

The second case is a 72 years old man, negative for human immunodeficiency virus (HIV) antibodies, admitted to the hospital for progressive worsening of neurological symptoms, i.e. vision changes, dysphasia and amnesia, started two months earlier. At admission, the spontaneous speech was fluent, but paraphasic and physical examination disclosed mild disorientation in space, ideomotor apraxia, right hemianopia, and mild right hemiparesis. He had surgery for a rectus adenocarcinoma 6 years before, locally recurred after 5 years. In the last hospital stay, he was treated with radiotherapy and chemotherapy with 5-fluorouracil and scanned by total-body computed tomography (CT) with a negative report for local, nodal or other organ involvement. He was affected by Franklin's disease diagnosed two years before with no underlying lymphoproliferative or autoimmune disorder. A brain magnetic resonance imaging (MRI) performed three weeks prior to the admission displayed two subcortical lesions, hyperintense in T2-weighted images and hypointense in T1-weighted images, without contrast enhancement or mass effect, in the left posterior temporal and in anterior parietal lobes, without restricted diffusivity on apparent diffusion coefficient maps. Spectroscopic analysis of the lesions showed no alterations. Laboratory tests, including blood leukocyte count and CD4+ to CD8+ cells ratio, were within the RI, but a severe deficiency of serum IgA (0.39 g/L, RI 0.70-4.00) and IgM (<0.19 g/L, RI 0.40-2.30) concentrations and a mild IgG deficiency (6.45 g/L, RI 7.00-16.00) were found. Serum IFE showed two γ-heavy chain MCs, migrating one in β and the other in γ-region, not associated with corresponding light chain; urine IFE interestingly showed a typical pattern with broad band of incomplete γ-heavy chain (Figure 2). Cerebrospinal fluid (CSF) evaluation was performed showing a mild increase in serum to CSF albumin ratio without neither total protein and cells increase nor oligoclonal band evidence (Figure 2). However, the same γ-heavy chain not associated with

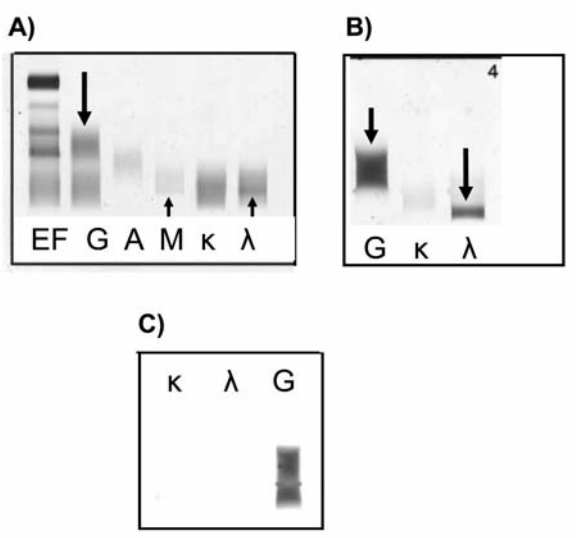


Figure 1
Immunofixation of serum and urine of the patient 1 affected by chronic lymphocyitic leukemia.
A) serum: the big arrow in β-region shows an abnormal broad band of γ-heavy chain, not associated with corresponding light chain; small arrows in γ-region show the IgMλ monoclonal component; B) urine: focusing on β-region of the agarose gel slide, the same broad band of unbound γ-heavy chain found in serum is shown (left); on γ-region also monoclonal free λ light chain (Bence Jones proteinuria) is found (right); C) urine after overnight incubation with anti-κ and λ antiserum: on the slide only reaction with anti-γ heavy chain occurred, confirming the presence of free heavy chains not reacting with the antiserum during the incubation.

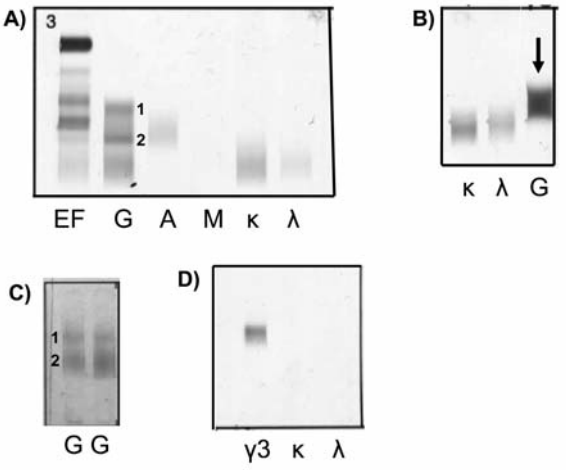


Figure 2
Immunofixation of serum, urine, and cerebrospinal fluid of the patient 2 affected by progressive multifocal leukoencephalopathy. A) serum: presence of two incomplete γ-heavy chain monoclonal components migrating in β- and γ-region; B) urine: typical γ-heavy chain disease pattern, with a broad band of incomplete γ-heavy chain migrating within β- and γ-region; C) cerebrospinal fluid (left) and serum (right) immunofixed with anti-γ antiserum show the same pattern of two γ-heavy chain incomplete fragments (number 1 and 2); D) serum after overnight incubation with anti-κ and λ antiserum immunofixed with anti-γ3 antiserum: on the slide only reaction with anti-γ3 heavy chain occurred showing that free heavy chains are of IgG3 subclass.

corresponding light chain identified in serum was also found in CSF by IFE (Figure 2). Polymerase chain reactions (PCR) performed on CSF for major neurotropic viruses, i.e. herpes simplex virus, Epstein-Barr virus, varicella zoster virus, enteroviruses, and Jacob-Creutzfeld virus (JCV), did not show the presence and replication of any virus. Screening for paraneoplastic autoantibodies (anti-Hu, anti-Yo, anti-Ri, anti-amphiphisin, anti-collapsin response mediator protein 5, anti-Ma-1, anti-Ta) was negative as well. The patient rapidly worsened developing a Gerstmann syndrome, with right-left confusion, dyscalculia, dysgraphia, finger agnosia, and severe right hemiparesis. A second MRI showed occipital and frontal spreading of the lesions that remained asymmetrical with preferential involvement of left hemisphere. PCR analysis was repeated on CSF still resulting negative for JCV DNA. A brain biopsy of the temporal lesion showed demyelination of the neurons with abundant macrophage infiltration, the presence of astrocytes with big hypercromatic nuclei and some mitotic figures and of oligodendrocytes with dense ground-glass nuclei. PCR for JCV was positive on the brain tissue sample. To treat severe hypogammaglobulinemia, a high dose (2g/Kg/die) intravenous immunoglobulin (IVIg) therapy was administered. Moreover, the antiviral drug cidofovir was prescribed. However, the patient rapidly worsened and within four months after the onset of the symptoms died.

DISCUSSION

Both patient sera showed by IFE at least a γ-heavy chain MC without a corresponding κ or λ light chain. Diagnosis of HCD is commonly confirmed by the evidence of serum unbound heavy chain on the IFE performed with anti-Fab and anti-Fc antisera or by immunoselections technique, e.g. immunodiffusion on agar with antisera anti-κ and anti-λ that, however, is more difficult to interpret than IFE (5). Heavy chain fragments derive from each IgG subclass, but IgG3 subclass is over-represented relative to their frequency among intact human IgG proteins (1). The most common subclass in γ-HCD is IgG1, which occurs in 65% of cases. IgG3 has been identified in 27% of patients, IgG4 in 5%, and IgG2 in 3% (1).

CLL is the most common type of leukemia in the western world. The median age of presentation is between 65 and 70 years; CLL is extremely rare below the age of 30 years, even if 20-30% of patients are under the age of 55 years at disease presentation. The male/female ratio is 2:1 (6). An increase in both lymphoid malignancies and subclinical monoclonal B-cell expansion can be found in first and second degree relatives of patients with CLL (7). The diagnosis of CLL requires the presence of at least $5x10^9$ B lymphocytes/L in the peripheral blood. The clonality of the circulating B lymphocytes needs to be confirmed by flow cytometry (8). CLL might be suspected in otherwise healthy adults who have an absolute increase in the clonal B lymphocytes, but who have less than $5x10^9$/L B lymphocytes in the blood. However, in the absence of lymphadenopathy or organomegaly (as defined by physical examination or CT scans), cytopenias, or disease-related symptoms, the presence of fewer than $5x10^9$ B lymphocytes/L is defined as “monoclonal B-lymphocytosis”. Monoclonal B-lymphocytosis may progress to frank CLL at a rate of 1% to 2% per year (8). Typically, CLL cells express weak surface immunoglobulin that, according to the scoring system for the diagnosis of CLL, define the malignancy of disease. The levels of surface immunoglobulin, CD20 and CD79b, are characteristically low compared with those found on normal B cells. Each clone of leukemia cells is restricted to expression of either κ or λ immunoglobulin light chains. Flow citometry immunophenotyping should be evaluated in all cases with low lymphocyte counts and atipical lymphocyte morphology.

Lymphocytosis is currently described among hematologic abnormalities occurring in HCD patients and an occasional patient having clinical presentation with CLL was reported (1). So, the first case reported here corroborate the finding that CLL could be a lymphoproliferative disease associated with HCD at presentation. Whereas biclonal gammopathy has been reported to occur in approximately 1-3% of all patients with serum MC (9), the association between γ-HCD and another monoclonal gammopathy is much higher. Moreover, in a literature review of 56 patients with γ-HCD, nine patients (16%) had biclonal gammopathy (9); in another series of 23 patients with γ-HCD, 7% were found to have an IgMλ intact monoclonal immunoglobulin (3). Interestingly, the serum IFE of the first case also showed an IgMλ MC together with the unbound γ-heavy chain broad band. The urine IFE of the same patient showed an evident free λ MC (Bence Jones proteinuria) as already described in an occasional HCD patient at presentation (3). This is the first case in our knowledge in which both serum IgMλ MC and Bence Jones proteinuria are described in the same HCD patient at the time of diagnosis.

PML is a fatal demyelinating disease of the central nervouse system (CNS) caused by the infection of oligodendrocytes by polyomavirus JCV. It generally occurs in patients in whom a severe immunosuppression allows the virus to reactivate from its latent state and reach the CNS. Conditions more commonly associated with PML include lymphomas, HIV infection, autoimmune diseases and treatment with natalizumab in multiple sclerosis patients . This is the first description of PML associated to Franklin’s disease. At the state of the art, the natural history of γ-HCD is not completely defined, because only less than 200 cases of γ-HCD has been reported in the literature. Although cell-mediated immunity is considered to play an essential role to prevent JCV reactivation, we can infer that factors promoting PML, in our case report, were both hypogammaglobulinemia and the production of abnormal, not functional immunoglobulins. Recently, two patients affected by a common variable immunodeficiency with hypogammaglobulinemia who developed PML have been described (10). Moreover, in the same paper hypogammaglobulinemia has been reported to be a common finding in patients with hematological mali-

gnancies treated with different chemotherapic agents plus rituximab at the PML developing time. So, we hypothesize that a reduction of circulating immunoglobulins could be considered a powerful risk factor for PML. IgM and IgA can play a protective role as well as IgG, and this could explain the poor response to IVIg in the patient.

Our findings show how HCD diagnosis can be established by serum or urine IFE using specific antisera in combination with immunoselection technique in patients with different pathologies not usually associated to HCD. It could be useful to use this technique also in clinical cases in which γ- or other heavy chains may be found, e.g. in autoimmune associated diseases (like non-Hodgkin lymphoma) or not associated disease (monoclonal gammopathy of undetermined significance). Serum and urine IFE should be performed in all patients with a confirmed or clinically and electrophoretically suspected plasmacell lymphoproliferative disorder.

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