Case 6.1 Acute leukaemia (common type)

A 7-year-old boy presented with malaise and lethargy of 6 days duration. He had become inattentive at school, anorexic and had lost 3 kg in weight. On examination he was thin, anxious and clinically anaemic. There was mild, bilateral, cervical lymphadenopathy and moderate splenomegaly.

On investigation, he was pancytopenic with a low haemoglobin (80 g/l), platelet count (30×10^9/l) and white cell count (1.2×10^9/l). The blood film showed that most leucocytes were blasts; the red cells were normochromic and normocytic. Bone marrow examination showed an overgrowth of primitive white cells with diminished numbers of normal erythroid and myeloid precursors. Acute leukaemia was diagnosed.

The circulating blast cells were typed by immunological methods: they did not react with monoclonal antibodies to human T-cell precursor antigens (CD2, CD7), but they were positive for major histocompatibility complex class II (DR), common acute lymphoblastic leukaemia (CD10) and B-cell precursor (CD19) antigens, and contained the enzyme terminal deoxynucleotidyl transferase (Tdt) (see Table 6.2). The phenotype of the blasts was that of acute leukaemia of early precursor B cells (see Fig. 6.2), and the prognosis in this child was relatively good. Cytogenetics confirmed ETV6-RUNX1 mutation or t(12;21) translocation, indicating an good prognosis.

Table 6.2 Panel of antibodies for the diagnosis of acute leukaemias

	B lymphoid	T lymphoid	Myeloid	Non-lineage-restricted
First line	CD19, CD10 (early B cells), CD79a (BCR), CD24	CD2, CD3, CD4, CD5, CD8	CD117 (stem cell growth factor receptor), CD13 Anti-myeloperoxidase	Tdt
Second line	CD138	CD7	CD33, CD41, CD42, CD61	CD45

CD antigens are defined by monoclonal antibodies (see Chapters 1 and 19). ALL, Acute lymphoblastic leukaemia; Clg, cytoplasmic immunoglobulin; Slg, surface immunoglobulin; Tdt, terminal deoxynucleotidyl transferase.

Essentials of Clinical Immunology, Sixth Edition. Helen Chapel, Mansel Haeney, Siraj Misbah, and Neil Snowden.

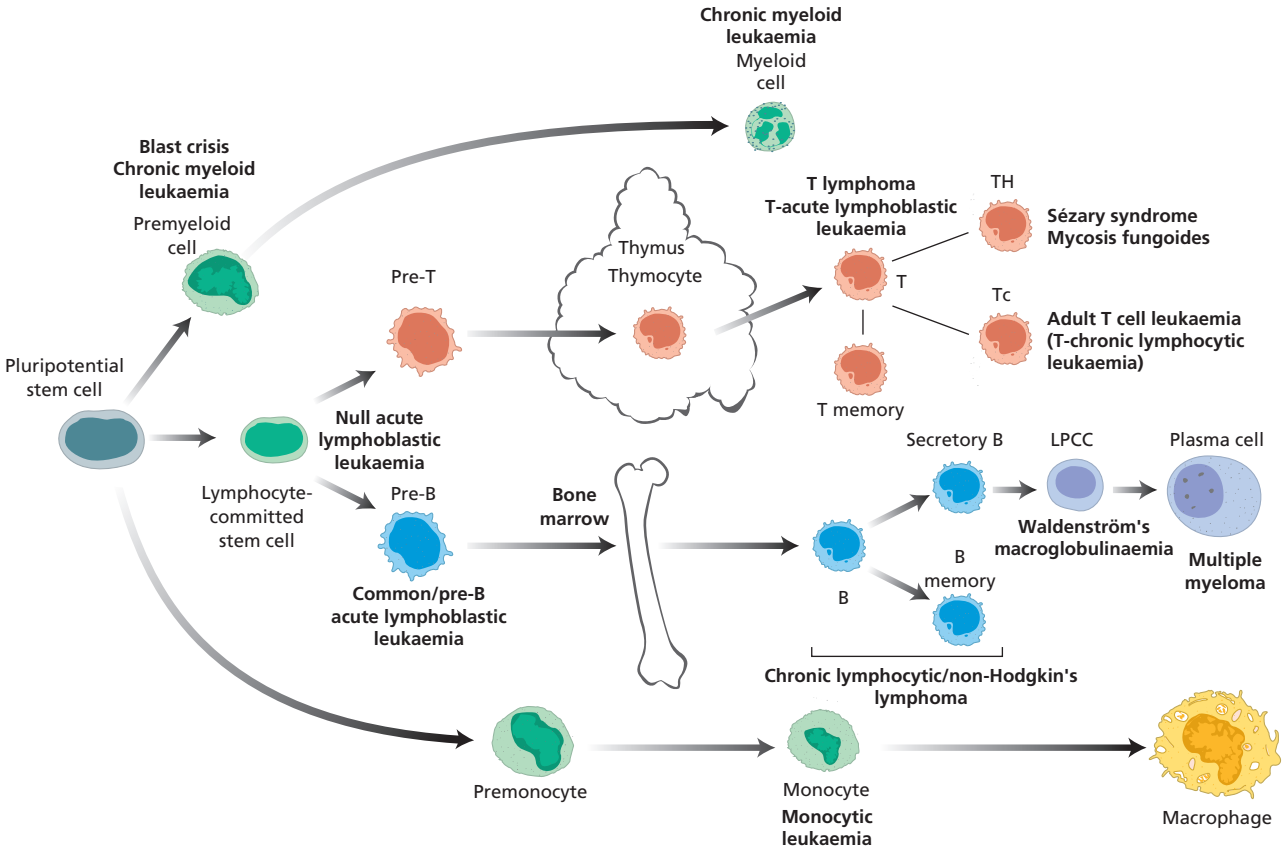
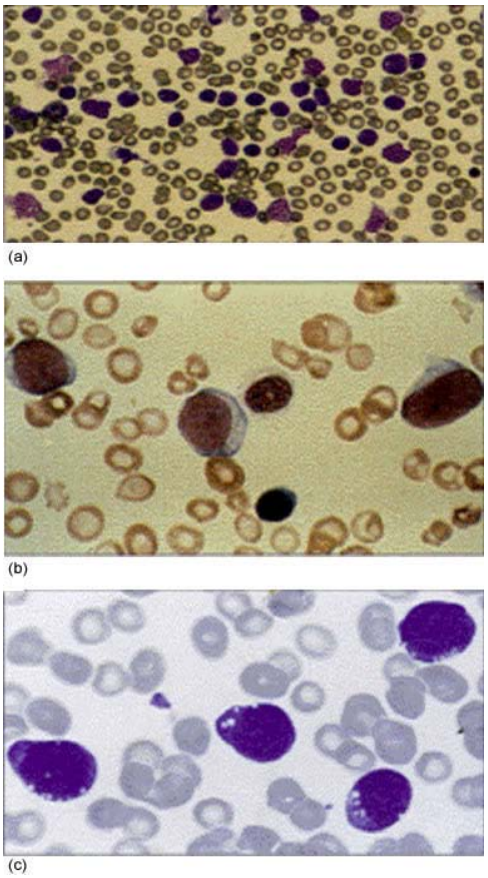
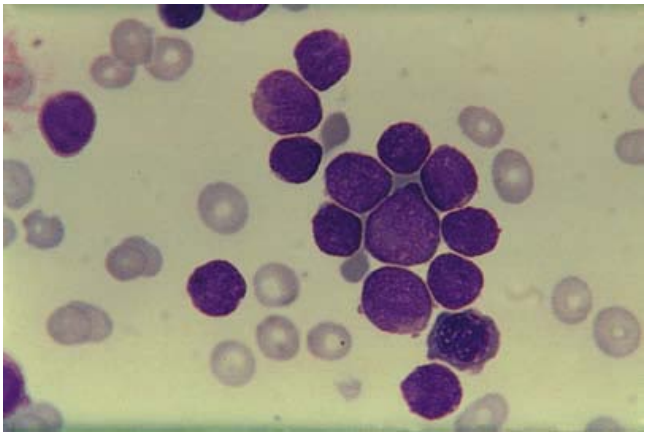


Fig. 6.2 Malignant counterparts at each step in the leucocyte differentiation pathway. LPCC, Lymphoplasma cytoid cell; T, T cell; TH, T helper cell; Tc, T cytotoxic cell; B, B cell.



Case Figure 6.1a Acute lymphoblastic leukaemia: (a) blood film showing blasts; (b) blasts in higher power (most common form) but in some patients the blasts have vacuoles or more abundant cytoplasm (c) – in this patient the red cells are microcytic.



Case Figure 6.1b Acute lymphoblastic leukaemia. The lymphoblasts are large with neucleoli. There is very little cytoplasm and no granularity.

Case 6.2 Chronic lymphocytic leukaemia

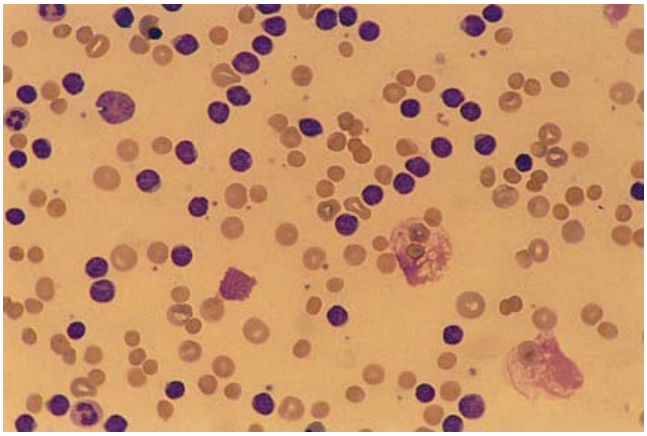
A 62-year-old man presented with general lethargy, night sweats and loss of weight. He had suffered five chest infections during the previous winter, despite being a non-smoker. On examination, there was moderate, bilateral cervical lymphadenopathy and left inguinal lymph node enlargement. The spleen and liver were enlarged 5 cm below the costal margins. There was no bone tenderness and there were no lesions in the skin. On investigation, his haemoglobin was slightly low, (10.2 g/l) the platelet count (251×10^9/l) was normal but his white cell count was increased to 150×10^9/l; the film showed that 98% of these were small lymphocytes.

The features on the blood film were suggestive of chronic lymphocytic leukaemia and immunophenotyping confirmed this diagnosis. Ninety per cent of the cells were B cells ($CD\ 19^+$); they all expressed CD 19 and CD5 confirming the diagnosis (Table 6.4). The serum immunoglobulins were low: IgG 2.2 g/l (NR 7.2–19.0 g/l); IgA 0.6 g/l (NR 0.8–5.0 g/l) and IgM 0.4 g/l (NR 0.5–2.0 g/l). There was no monoclonal immunoglobulin in the serum or the urine.

Table 6.4 Immunophenotyping in differential diagnosis of chronic lymphocytic leukaemias

	Lymphocyte markers*			
Disease	**CD3**	**CD19**	**CD5**	**CD11c**
Normal pattern	75	12	2	0
Chronic lymphocytic leukaemia	10	90	90	0
Sézary syndrome	92	2	0	0
Hairy cell leukaemia	10	60	0	60

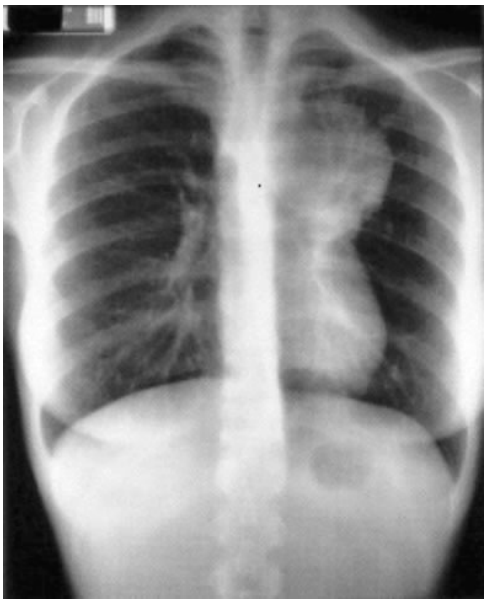
*Results expressed as percentage of peripheral blood lymphocytes positive for marker.



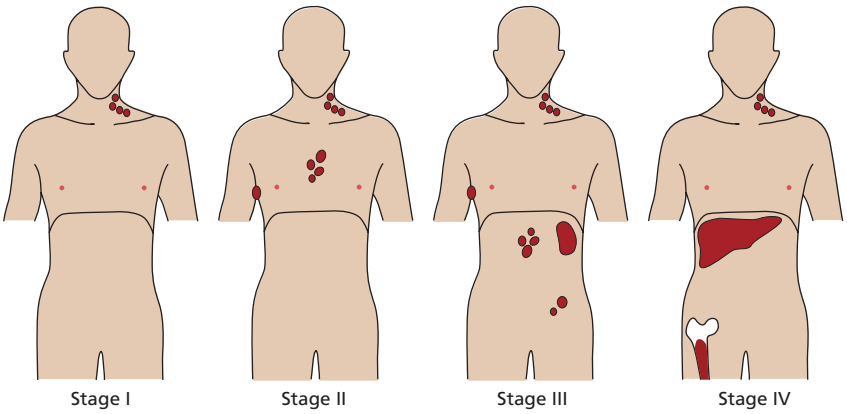
Case Figure 6.2 Chronic lymphocytic leukaemia. There is a predominance of mature lymphocytes These have very little cytoplasm and no nucleoli. There are a few smear cells. There is a degree of polychromasia (odd shaped red cells) due to a reticulocytosis in the presence of autoimmune haemolysis (not present in this Case Figure).

Case 6.3 Hodgkin's disease

A 23-year-old man presented with malaise, night sweats, loss of weight and intermittent fever dating from a flu-like illness 3 months previously. On examination, he had bilateral cervical and axillary lymphadenopathy; the glands were 2–5 cm in diameter, firm, rubbery, discrete and fairly mobile. His liver and spleen were not enlarged. Investigation showed that his haemoglobin was low (113 g/l) and the white cell count was normal (4.2×10^9/l) but his erythrocyte sedimentation rate (ESR) was raised at 78 mm/h; the blood film did not show any abnormal cells. No enlargement of the hilar glands was seen on chest X-ray. A cervical lymph node was removed for histology. The gross architecture of the node was destroyed; the tissue consisted of histiocytes, eosinophils, lymphocytes and giant cells known as Reed–Sternberg cells. These large binucleate cells are characteristic of Hodgkin's disease. Bone marrow examination was normal apart from a reactive eosinophilia and computed tomography (CT-PET) showed no involvement of other lymph nodes. This patient had stage 2 Hodgkin's disease, because, although only lymphoid tissue above the diaphragm was involved, his ESR was above 40 mm/h. In view of his symptoms, the suffix 'B' was added to the stage, suggesting a poorer prognosis associated with systemic symptoms. He was given cytotoxic chemotherapy with ABVD.



Case Figure 6.3a Chest X-ray in another patient with Hodgkin's disease – showing enlarged mediastinal lymph nodes and infection in lung bases.



Case Figure 6.3b Staging of Hodgkin's disease – in addition to staging by involved sites (as here), A or B are added (B indicates systemic symptoms e.g. fever, night sweats).

Case 6.4 Non-Hodgkin's lymphoma

A 59-year-old man presented with a gradually increasing lump in his right groin of 6 months' duration, which he thought was a 'hernia'. This was a large inguinal lymph node. He had suffered repeated urethritis in the past. He had no other symptoms, but was found on examination to have splenomegaly (7 cm below the costal margin) without hepatomegaly.

On investigation, his haemoglobin was low (118 g/l) but his white cell count and differential were normal. His ESR was 58 mm/h and the lactate dehydrogenase level was also high. His serum immunoglobulins were all reduced: his IgG was 5.2 g/l (NR 7.2–19.0 g/l); IgA 0.3 g/l (NR 0.8–5.0 g/l); and IgM 0.3 g/l (NR 0.5–2.0 g/l). Serum electrophoresis showed no monoclonal bands. The lymph node was excised; light microscopy showed irregular follicles with mixtures of small and large cells throughout but no organized germinal centres. Reactive follicular hyperplasia was a possibility but immunophenotyping of tissue sections showed monoclonality, with strong cellular staining of the cells in the multiple follicles with anti-IgG and anti-k monoclonal antibodies. Normal interfollicular T-cell staining was present. This patient had a follicular type of NHL and cytogenetic analysis of the lymph node revealed chromosomal translocations associated with aggressive disease.

Case 6.5 Benign paraproteinaemia

A 49-year-old woman presented with a 6-month history of vague aches and pains in her chest. On examination, she was overweight but had no abnormal physical signs.

Her haemoglobin was 136 g/l with a white cell count of 6.7×10^9/l and a normal differential. Her ESR was 34 mm/h. Tests of thyroid function were normal. However, protein electrophoresis showed a small paraprotein band in the γ band; this band was an IgG of γ type. Her serum IgG raised at 20.1 g/l (NR 7.2–19.0 g/l), with an IgA of 1.9 g/l (NR 0.8–5.0 g/l) and an IgM of 3.0 g/l (NR 0.5–2.0 g/l). Electrophoresis of concentrated urine showed no monoclonal light chains and the plasma ratio of free kappa:lambda light chains was normal. The serum paraprotein measured 10 g/l by densitometry (Chapter 19). A bone marrow examination showed only 2% plasma cells. Together with the absence of osteolytic lesions, the absence of monoclonal free light chains in the urine and normal serum IgA and IgM levels, these findings supported a diagnosis of benign monoclonal gammopathy, also known as a monoclonal gammopathy of unknown significance (MGUS). This woman has been followed at 6-monthly intervals for 22 years with no increase in the paraprotein level. She will continue to be seen at yearly intervals.

Case 6.6 Multiple myeloma

A 66-year-old man presented with sharp, constant, low back pain, dating from a fall from a ladder 6 weeks earlier. On direct questioning, he did admit to vague malaise for over 6 months. On examination, he was in considerable pain but otherwise seemed fairly fit. He was mildly anaemic but had no lymphadenopathy and no fever. There were no signs of bruising, no finger clubbing, no hepatosplenomegaly and no abdominal masses. On investigation, his haemoglobin was low (102 g/l) but his white cell count was normal (6.2×10^9/l). He had a normal differential white cell count and a normal platelet count but his ESR was 98 mm/h. Total serum proteins were raised at 98 g/l (NR 65–75 g/l) and his serum albumin was low; plasma creatinine and urea were normal. He had a raised serum calcium level (3.2 mmol/l) but a normal alkaline phosphatase. Serum protein electrophoresis revealed a monoclonal band in the γ region (Fig. 19.3), with considerable immunosuppression of the rest of this region (see Fig. 6.11). The band was typed by immunofixation Figures 6.11 and shown to be IgA of κ type. Quantification of serum immunoglobulins showed a low IgG of 6.0 g/l (NR 7.2–19.0 g/l), a high IgA of 15.3 g/l (NR 0.8–5.0 g/l), and a low IgM of 0.2 g/l (NR 0.5–2.0 g/l). Electrophoretic examination of concentrated urine showed a monoclonal band in the α region, which was composed of free κ light chains. X-rays of his back showed a small, punched-out lesion in the second lumbar vertebra but a subsequent skeletal survey did not show any other bone lesions. Bone marrow examination showed an increased number of atypical plasma cells; these constituted 45% of the nucleated cells found on the film. This man showed the features required for a diagnosis of multiple myeloma (see Box 6.5).

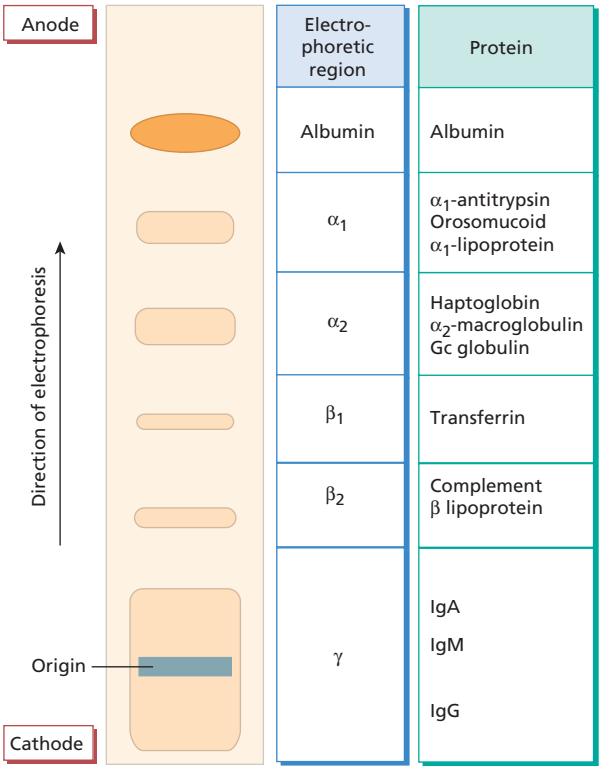


Fig. 19.3 Principle of serum protein electrophoresis. At the pH of routine electrophoresis (pH 8.6), serum proteins carry a net negative charge and migrate towards the anode. Some weakly charged proteins, such as immunoglobulins, are carried back towards the cathode by the flow of buffer.

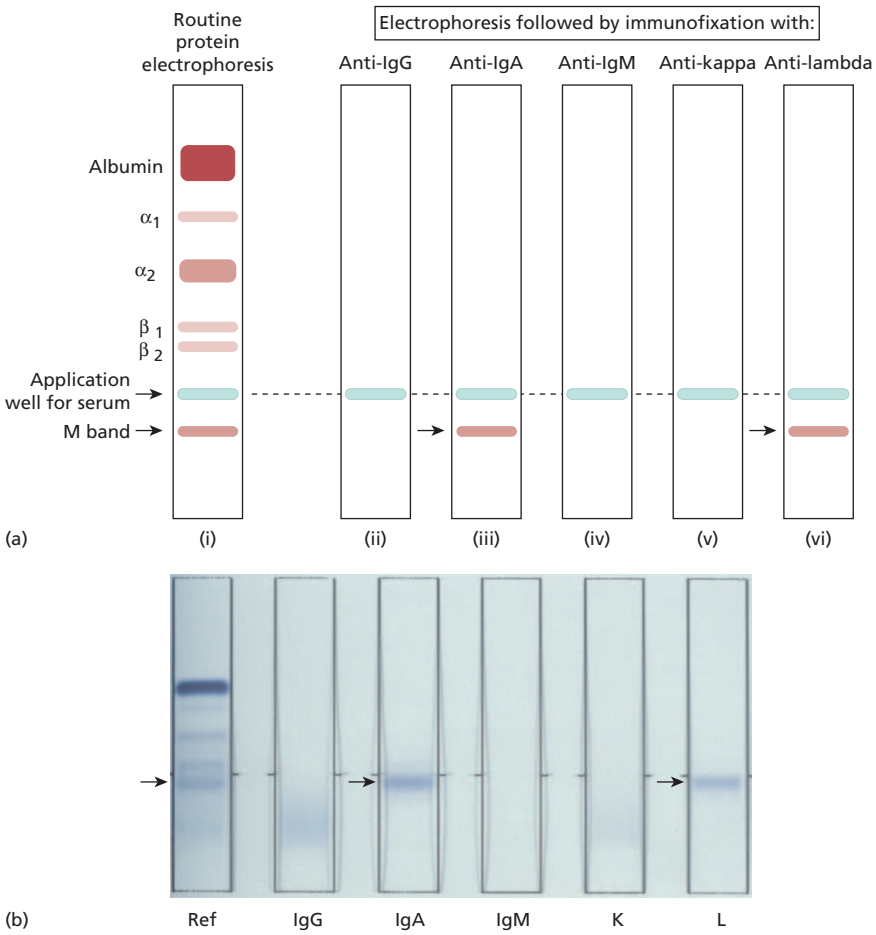
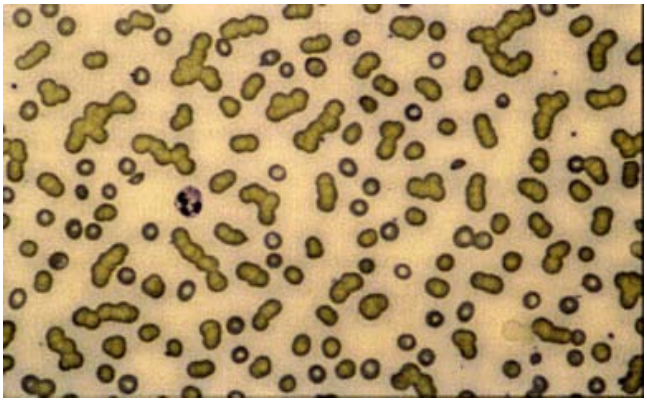
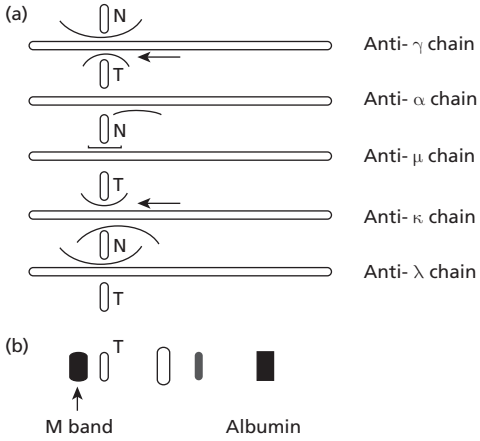


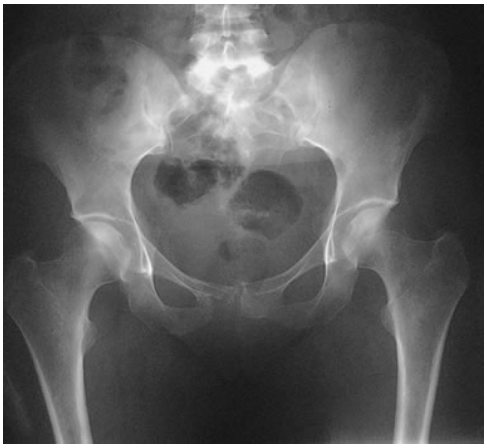
Fig. 6.11 Typing an M band by immunofixation. In this schematic example (a), the M band found on electrophoresis (i) is identified as an IgA (type λ) as shown on the actual fixation gel (b).



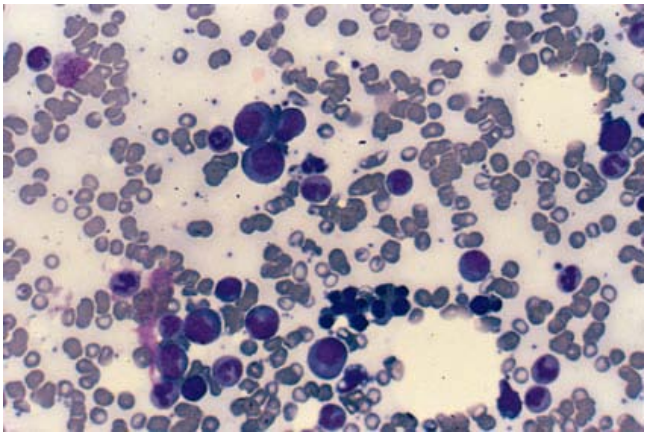
Case Figure 6.6a Multiple myeloma – blood film usually shows 'rouleaux' formation and background staining due to excess protein of monoclonal Ig as seen here.



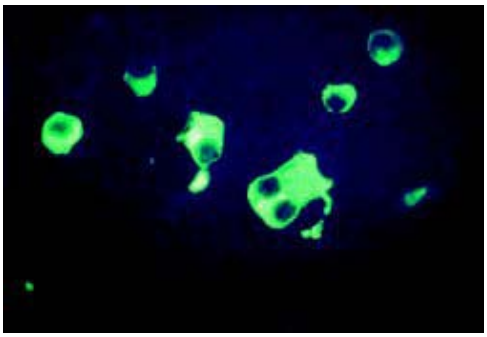
Case Figure 6.6b (a) Immunoelectrophoresis of test (T) and normal (N) serum. The anode is to the right. After electrophoresis, the troughs were filled with antisera to gamma chain, alpha chain, mu chain, kappa light chain and lambda light chain. A monoclonal IgG (kappa type) is seen as a localized, thickened bowing of the relevant precipitin lines (arrowed). The test serum has no detectable IgA or IgM by this method. (b) Serum protein electrophoresis shows separation into albumin alpha1, alpha2, beta1 and gamma-globulin regions. The test serum (T) shows a discrete M band (arrowed) in the gamma region.



Case Figure 6.6c Myeloma of bone. Note the symphysis pubis has been eroded by myeloma. There are no apparent deposits in the upper femur or pelvis.



Case Figure 6.6d Myeloma cells. This bone marrow shows a number of different types of cell. The larger cells with accentric nuclei and basophilic cytoplasm are myeloma cells. Note the perinuclear transparency which represents the Golgi apparatus.



Case Figure 6.6e Bone marrow – direct immunofluorescent staining with anti-lambda antibody shows cytoplasmic staining in atypical plasma cell. There is no staining with anti-kappa antibody, i.e. all the plasma cells are monoclonal.

Case 6.7 Waldenströms macroglobulinaemia

A 76-year-old woman presented with a 6-month history of weakness, malaise, exertional dyspnoea and abdominal discomfort. In the previous month she had experienced epistaxes and headaches but did not have visual disturbances, weight loss, bone pain or recurrent infections. On examination she was pale, with moderate axillary and cervical lymphadenopathy. Her liver and spleen were enlarged.

On investigation, she had an ESR of 112 mm/h and a haemoglobin of 108 g/l. Her white cell count and differential were normal. The total serum protein was increased to 130 g/l. Protein electrophoresis (Chapter 19) and immunofixation (Chapter 19) showed a dense paraprotein in the γ region which proved to be an IgM of κ type. Quantification of the serum immunoglobulins showed normal IgG (9.4 g/l) and IgA levels (1.1 g/l), but her IgM was markedly raised at 64.5 g/l (NR 0.5–2.0 g/l). By densitometry (Chapter 19), the paraprotein (see Fig. 19.5) measured 63 g/l. Electrophoresis of concentrated urine showed no free monoclonal light chains and there were no bone lesions on X-rays of her chest and skull. Her serum viscosity, relative to water, was 4.7 (NR 1.4–1.8). A bone marrow examination showed a pleomorphic cellular infiltrate composed of a mixture of small lymphocytes, plasma cells and cells of an intermediate appearance, called lymphoplasmacytoid cells. These are features of Waldenström's macroglobulinaemia.

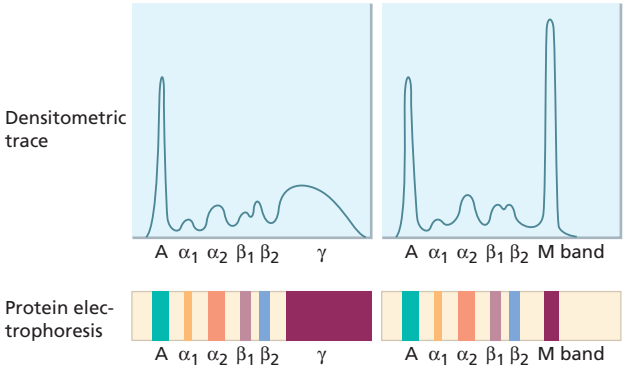


Fig. 19.5 Densitometric analysis of protein electrophoresis for quantification of an M band. A, Albumin. Normal trace on left.