

Historical Review

HISTORICAL ASPECTS OF CHRONIC LYMPHOCYTIC LEUKAEMIA

In the beginning

As far as we know, leukaemia has always existed. Probably the first patient noticed by a doctor as exhibiting the symptoms was a Monsieur Vernis, a 63-year-old Parisian lemonade salesman (Velpeau, 1827). We know that in this profession and in his former job as a florist he had been a happy, carefree individual with an eye for the ladies; yet he had managed to avoid the ravages of syphilis. Alas, despite Dr Velpeau's eminence (Fig 1), nobody else seemed interested in the disease. It took a controversy over precedence for leukaemia to reach the medical agenda in earnest. In the same issue of the Edinburgh Medical and Surgical Journal in October 1845, two case reports (Bennett, 1845; Craigie, 1845) described patients who probably had leukaemia. Craigie's patient, a 30-year-old man whose illness began in 1841, had a spleen that weighed seven pounds three and a half ounces and 'pink, wine-lee-coloured, gromous blood mixed with whitish-coloured masses of purulent lymph'. 'Gromous' means viscous or jelly like. The patient survived for 1 year with deteriorating fatigue, weakness and increasing abdominal girth and pain. It is quite likely that this was the first reported case of chronic myeloid leukaemia.

We do not know for sure what type of leukaemia John Hughes Bennett, the Englishman later to become Professor of Medicine in Edinburgh, reported. His patient, John Menteith, a 28-year-old slater from Edinburgh, had been aware of a mass in the left side of his abdomen for 8 months before he died. At post-mortem examination, he had massive enlargement of his liver, spleen and lymph nodes. Examination of his blood revealed 'the existence of true pus, formed universally within the vascular system, independent of any local purulent collection from which it could be derived'. Although the involvement of the lymph nodes suggests chronic lymphocytic leukaemia (CLL), it is more likely that a patient of this age would have had a spill-over lymphoma.

We cannot fully diagnose the case of Marie Straide, reported only 6 weeks later by Rudolph Virchow in Berlin (Virchow, 1845), either. Marie was a 50-year-old cook, who died with a huge spleen 6 months after presentation. In her blood, the ratio of pigmented to colourless corpuscles was reversed. As she developed furuncles and suppurations of the skin and had several nosebleeds during her short illness, she may well have had acute leukaemia.

The word 'leukaemia' (leukämie) was coined by Virchow (1847a), and by that time he had already published a further nine cases (Virchow, 1846, 1847b). Meanwhile, Bennett preferred the term 'leucocythaemia' and collected a further 35 cases that he salami-sliced into four papers and a monograph (Bennett *et al*, 1851a,b,c,d, 1852). Most of the early cases had splenomegaly as a major feature, although at least one of Virchow's cases had generalized lymphadenopathy without splenomegaly (Virchow, 1846), perhaps the first true case of CLL. Thereafter, Virchow classified leukaemia into 'splenic' and 'lymphatic' forms, recognizing that splenic leukaemias had granular leucocytes with trefoil-like nuclei in contrast to the agranular leucocytes with smooth round nuclei of the lymphatic leukaemias (Virchow, 1851).

It is important to grasp the atmosphere as the two competing doctors strove for pre-eminence in the field of leukaemia. Even the name of the condition was acrimoniously disputed.

Bennett (Fig 2) was 33 years old in 1845. He had graduated from Edinburgh after a brilliant student career. He was apprenticed to a surgeon in Maidstone and he then spent 2 years in Paris under the great microscopist Donné, who himself has some claim to have described the pathological features of leukaemia before either Virchow or Bennett (Donné, 1844). This case had presented clinically in 1839, and it is easy to believe it had been much talked about by the doctors there. After Paris, where he founded and was first President of the Parisian Medical Society, Bennett spent a further 2 years in Germany and then returned to Edinburgh. Here, he gathered a reputation as an outstanding teacher. By 1845, he was already a Fellow of the Royal Society of Edinburgh, and in 1848 was elected to the Chair at the Institutes of Medicine. That he never achieved the much more prestigious Chair of Physic at Edinburgh University has been attributed to his short temper, pugnacious attitude and certainty of his own virtues. Even the writer of his obituary had to admit that 'his tendency to indulge freely in critical and sarcastic remarks upon the works of others did not make him a general favourite with some of his professional brethren' (McKendrick, 1875).

Virchow (Fig 3) was only 24 in 1845 and just 2 years out of medical school. The Berlin Army Medical School (Friedrich-Wilhelms Institut) must have been a very much more disciplined establishment than Edinburgh, but Virchow was just as opinionated as Bennett. Part of the requirements for his entry to medical school was an undertaking to serve in the army on qualifying. When

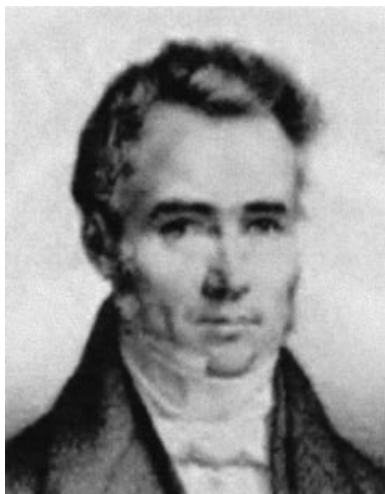


Fig 1. Armand Velpeau, 1795–1867. Was his the first report of a case of CLL? Picture from US National Library of Medicine.

sent to report on an epidemic of typhus in 1847, his experiences led him to become a politically active radical and his part in the uprising in 1848 caused his expulsion from the Charité where he held his academic position. He was elected to the Berlin City Council in 1859, where he



Fig 2. John Hughes Bennett, 1812–1875. Like many since that time, he did most of his best work as a young man. Picture from library of Royal College of Physicians of Edinburgh.



Fig 3. Rudolph Virchow, 1821–1902. Virchow was only 24 when he described his first case of leukaemia. Picture from US National Library of Medicine.

instituted many public health improvements. Later, as a member of the Prussian Lower House, he opposed Bismarck and became famous for his radical views and polemical speeches. In the Franco-Prussian war, he organized the ambulance service of the German army.

Incontrovertibly the greatest pathologist of his generation, perhaps of all time, Virchow became assistant to Professor Froriep at the Charité in Berlin and was given the task of investigating the inflammation of veins. Froriep had been in Paris at the same time as Bennett, so might well have known of Donné's case.

The first ante-mortem diagnosis of leukaemia was made by Fuller (1846). Both Virchow and Bennett made their discoveries at the autopsy table. How sad it is that so few young doctors are to be found there these days. Despite their dispute, both Bennett and Virchow agreed that the main trouble lay in the enormous number of colourless corpuscles in the blood. Without the ability to stain blood cells, their ideas on pathogenesis were exotic. Bennett believed that the red cells were the extruded nuclei of white cells and that a failure of this process led to the reversal of the ratio of the two types of cell. Virchow believed that the leukaemic cells came from the lymph, although he conceded that the spleen was an alternative source.

The bone marrow. Ernst Neumann first recognized the central role of the bone marrow in leukaemia (Neumann, 1870). Neumann, like Immanuel Kant a century before, was a lifelong citizen of Königsberg. He gave us our conception of the marrow as the source of the blood cells, eventually achieving fame as a visionary man of science and a writer of impeccable German. Meanwhile, he had to endure 20 years of ridicule by the medical establishment, who clung to the outmoded ideas of the previous generation. Nothing changes. Neumann recognized two patterns

of bone marrow involvement in leukaemia: pyoid hyperplasia, dominated by highly granular cells, and lymphadenoid hyperplasia, where the cells had pale homogeneous nuclei and were almost devoid of cytoplasm (Neumann, 1878).

Stained cells. Paul Ehrlich (Fig 4) developed a tri-acid stain that allowed the clear definition of nucleus, cytoplasm and other cytoplasmic detail (Ehrlich, 1891). His cousin, Carl Weigert, already a master at staining tissues, guided him. The German chemical industry had recently discovered the aniline dyes – a prime example of technology driving science. Ehrlich was a man obsessed with his studies. His skin and clothing were stained from his experiments, as was his billiard table where he regularly conducted them. He frequently moved his base; from Leipzig to the Charité in Berlin, to Koch's Institute, to Steglitz and to Frankfurt. Although he was showered with honours, including the Nobel Prize in 1908, his last years were unhappy as he was involved in controversy over his introduction of the arsenicals for the treatment of syphilis. Nevertheless, Ehrlich's stains allowed the leukaemias to be more clearly separated, and by the turn of the century Türk (1903) published criteria for the diagnosis of CLL.

Overlap with lymphoma. In this same publication, Türk stressed the resemblance between CLL and lymphoma. Thomas Hodgkin had first described fatal tumours of the lymph nodes, although in retrospect we now know that only three of his seven patients actually had Hodgkin's disease (Hodgkin, 1832). Virchow (1863) described lymphosarcoma as a malignant tumour of the lymphoid tissue distinct from leukaemia and tuberculosis. Kundrat (1893) used the same term, recognizing that the disease spread between different groups of lymph nodes but spared the blood and bone marrow, thus distinguishing it from leukaemia. However, Türk (1903) pointed out that transitions between



Fig 4. Paul Ehrlich, 1845–1915. Stains laid the foundation. Picture from US National Library of Medicine.

lymphosarcoma and CLL did occur and he regarded them as part of a family of diseases. A lively debate between these two extreme positions continued well into the twentieth century and even today there remains some difficulty in distinguishing CLL from some forms of lymphoma.

The twentieth century

Minot (Fig 5) and Isaacs produced a detailed description of the clinical features and natural history of 80 cases of CLL, and pointed out that radiation therapy shrank the lymph node masses but did nothing for the course of the disease (Minot & Isaacs, 1924). Over the next few decades, many doctors studied CLL, but there were very few insights into its nature. Richard Doll established that it was a disease of late middle age, twice as common in men (Court Brown & Doll, 1959). The usefulness of chlorambucil (Galton *et al*, 1955) and corticosteroids (Shaw *et al*, 1961) in treatment was recognized. By the late 1960s, three great haematologists knew all there was to know about the clinical features of CLL and its natural history. The large series of patients from Maxwell Wintrobe's department at Salt Lake City confirmed the very variable survival times in this disease and suggested a means of stratifying the disease according to its clinical features (Boggs *et al*, 1966). David Galton described a proliferative variant that did poorly and a stable variant that did well (Galton, 1966). He also described CLL as a disease of accumulation of long-lived functionally incompetent lymphocytes, a conclusion arrived at independently by William Dameshek (Dameshek, 1967). In 1973, Mørk Hansen published a series of 189 cases of CLL that had been followed for a long period of time (Hansen, 1973). This volume, which describes CLL in great detail, is one of my treasured possessions and has been of great value to me in preparing this review. However, because no one had a clear idea of what a lymphocyte did, further progress was inhibited.



Fig 5. George Richards Minot, 1885–1950. Nobel Laureate in 1934. His was the first large series of cases. Picture from US National Library of Medicine.

What is a lymphocyte?

A major textbook of immunology published in the mid-1950s contains only one reference to the lymphocyte and that was to dismiss it as a serious contender as an antibody-producing cell (Boyd, 1956). Although immunology had made great strides after Jenner's rather pragmatic approach to vaccination through the work of Pasteur, Ehrlich, Landsteiner and Metchnikov, it was still all about macrophages and antibodies. As a young medical student in Bristol in the 1960s, I was taught by Professor Yoffey that the lymphocyte was the precursor of the red cell (Yoffey, 1966).

Cell-mediated immunity. The idea that the lymphocyte was in some way involved in the immune response kept surfacing. James B. Murphy, working mainly alone at the Rockefeller Institute in New York on what he thought was tumour immunity but in fact was probably transplantation immunity, assembled an impressive array of evidence (Murphy, 1926). He found that exposure of rodents to X-rays killed their lymphocytes and lowered resistance to cancer grafts. Murphy also discovered that tumours can be grafted into embryos, but are rejected if a graft of spleen or marrow from an adult is included. Finally, he found that rejection of a tumour graft is accompanied by a proliferation of lymphocytes in spleen and bone marrow and that these invade the tumour.

In the 1940s and 1950s, Peter Medawar and colleagues (Billingham *et al.*, 1954), inspired by the horrific burns suffered by wartime fliers, worked on skin grafts. They demonstrated that the accelerated rejection of second grafts could be transferred by lymphocytes and not by antibody. Even Landsteiner, shortly before his death, had demonstrated that contact sensitivity could be transferred between animals by lymphocytes (Landsteiner & Chase, 1942). Thus, it was fairly easy to accept that the lymphocyte might be responsible for what became known as cell-mediated immunity.

Antibody. Antibody was a different proposition. Although McMaster & Hudduck (1935) had shown that most of the antibody produced in response to injection of antigen into the ear of an animal was produced in draining lymph nodes, it was clear that the antigen was picked up there by macrophages. The simplest explanation was that they also made the antibody. However, several workers noticed that such an immune response made no alteration to the macrophage, yet caused the proliferation of the lymphocytes, which became big and blastic. Ehrlich and Harris (Harris *et al.*, 1945) in Philadelphia cannulated the efferent lymphatic of a responding lymph node and demonstrated that the antibody contained in the lymphocytes that they collected was six times the concentration of that in the fluid.

The plasma cell. Better evidence that the plasma cell was the main antibody-producing cell was emerging from Scandinavia. First, the observation that myeloma, a tumour of plasma cells, was associated with an excess of antibody globulin (Bing & Plum, 1937), then the observation that repeated immunization of rabbits led to marked increases of plasma cells in lymph nodes and bone marrow (Bjørneboe & Gormson, 1943) and, finally, the convincing evidence of the

binding of fluorescence-labelled antigen to plasma cells and not lymphocytes by the Harvard workers (Coons *et al.*, 1955).

Ehrlich and Harris were forced to concede the primacy of the plasma cell, but few scientists are willing to let go of their ideas (and research grants) so easily. In a series of brilliant experiments, Harris and his wife explored the immune response in the rabbit to *Shigella* bacilli. They demonstrated that lymphocytes taken from a responding lymph node, washed free of antigen and macrophages, would induce the production of antibody when injected into a different animal, presumably by transmigration into plasma cells (Harris & Harris, 1960). They were right, but the sceptics still deemed convincing.

Lymphocyte life span. The final obstacle to proving that the lymphocyte had anything to do with immunity was its fast disappearance time. At the beginning of the century, Davis & Carlson (1909) in Chicago demonstrated that the blood lymphocytes were replaced four times every 24 h. Of their four possible explanations, their first hypothesis (that they were rapidly destroyed) was more easily believed than their last explanation (that they escaped through capillary endothelium and then recirculated via the lymphatics), which happened to be the correct answer. The essence of immunity is memory. For the lymphocyte to play a part in immunity it must live long enough to hold a memory. James Learmonth Gowans, in the Dunn School of Pathology at Oxford, solved the conundrum. He injected radiolabelled lymphocytes into the bloodstream and collected them a few hours later from the thoracic duct (Gowans, 1959). When it became possible to examine the chromosomes of lymphocytes after stimulation by phytohaemagglutinin, it became apparent that treatment of men with ankylosing spondylitis by radiotherapy induced unstable chromosome aberrations. By studying these, cytogeneticists in Edinburgh were able to conclude that the average small lymphocyte went several years between cell divisions (Buckton *et al.*, 1967). The lymphocyte clearly lives long enough to carry memory.

Thymus and bursa. Beard (1900) in Edinburgh believed that the thymus was the source of all white blood cells, but that this function ceased early in life after the whole body had been seeded. After this the thymus could be removed with impunity. The obvious test of this hypothesis, to remove the thymus immediately after birth, was delayed for 60 years. Jacques Miller, working at the Chester Beatty Institute, discovered that neonatally thymectomized mice had impaired immune responses (Miller, 1961). Workers at Yale demonstrated that this deficiency involved delayed hypersensitivity and graft rejection, rather than antibody production (Arnason *et al.*, 1962).

The bursa of Fabricius is a lymphoid organ located at the dorsal aspect of the cloaca in birds. Like the thymus, it involutes rapidly after hatching. The obvious experiment of removing it shortly afterwards occurred to immunologists rather earlier than it did for the thymus. Chicks bursectomized shortly after hatching grew up to be chickens, but when (because of a shortage of birds for teaching) they were used in a class exercise they unexpectedly failed to produce antibody against *Salmonella* (Glick *et al.*, 1956).

This theme of two types of lymphocyte being processed by different neonatal organs to constitute the two arms of the immune response had great power and symmetry. Encouragingly, the experimental work in rodents and poultry was mirrored by the clinical studies of Bob Good in Minneapolis on immunodeficient children (Good & Varco, 1955). Thus, we had B cells and T cells. Much time and effort has been expended looking for a non-existent 'bursa equivalent' in mammals. Eventually, it was decided that the bone marrow itself fulfilled the function, but it is a mistake to think of B cells as 'bone marrow derived' as both types of lymphocytes have their genesis in the bone marrow.

Recognizing B and T cells. It is difficult to convey the excitement of the period of the early 1970s when it became possible to recognize B and T cells in the peripheral blood. Listening to Martin Raff speaking with that extraordinarily attractive accent at the British Society for Immunology meetings describing anti-theta antibodies reacting with T cells and anti- μ antibodies reacting with B cells fired me with enthusiasm for that fusion of haematology and immunology that I have practised ever since.

Paul Ehrlich had postulated the existence of preformed receptors on the outer surface of cells that could interact specifically with foreign substances (Ehrlich, 1900). He suggested that when these were bound to the receptor the cell would be switched to producing more of the receptor that would be shed into the surrounding medium as antibody. McFarlane Burnet postulated that each lymphocyte was different, genetically predetermined to synthesize only one type of antibody molecule (Burnet, 1959). Thus, after contact with antigen, only those cells pre-programmed to produce an antibody with a complementary structure would be stimulated to proliferate and produce antibody-producing progeny, in effect a clone of the antigen-recognizing cell.

Sell & Gell (1965) in Birmingham had first shown that antibodies against immunoglobulin could induce blast transformation in some lymphocytes, implying that immunoglobulin was located on the surface of some lymphocytes, presumably as a receptor for antigen. In Ave Mitchison's laboratory at Mill Hill, Martin Raff and Roger Taylor demonstrated by immunofluorescent staining that this was indeed the case (Raff *et al.*, 1970). It was the common belief at the time that immunoglobulin, or perhaps a portion of the molecule, was also the antigen receptor in T cells. A simple experiment by Raff demonstrated the error. The theta antigen (later renamed Thy-1) is present in the brain, thymus cells and thymus-derived cells in the spleen and lymph nodes of mice (Reif & Allen, 1964; Raff, 1969). Raff (1970) showed that all lymphocytes expressed either theta or immunoglobulin, but never both.

This was all in mice, of course. Then, towards the end of 1971, a flurry of papers appeared. In successive weeks of the October *Lancet*, Gus Nossal from Melbourne using ^{125}I -labelled antibody (Wilson & Nossal, 1971) and John Holborrow from the Canadian Red Cross Memorial Hospital at Taplow in Middlesex using direct immunofluorescence (Papamichael *et al.*, 1971) reported that B cells could be detected in human peripheral blood. Both series included

patients with CLL among their subjects. In contrast with normals in whom only approximately 7% of lymphocytes expressed surface IgM, in CLL an average 89% of lymphocytes carried surface immunoglobulin. These studies were marred by non-specific binding of IgG and by the assumption that the T-cell receptor must also be immunoglobulin, but, nevertheless, it had at last been established that the CLL cell was a B cell. The Australian paper also showed that CLL cells had far less surface immunoglobulin than normal B cells.

Who got there first? Was this to be 1845 all over again? There were other contenders. Grey *et al.* (1971a) demonstrated surface immunoglobulin on the cells of 20 CLL patients only a month later in a paper published in the *Journal of Clinical Investigation*, and this paper was certainly submitted before either of the *Lancet* papers. Moreover, it had been published in abstract form five months earlier (Grey *et al.*, 1971b). Both Seligmann's (Preud'homme *et al.*, 1971) and Pernis's (Pernis *et al.*, 1971) papers were published in December and a more comprehensive study from Paris (Preud'homme & Seligmann, 1972) appeared 12 months later. It was probably Eva Klein who should be given the priority, as her single case report appeared the previous year (Johansson & Klein, 1970). What this shows is how pointless such squabbles are. This was an idea whose time had come. As the subject of much gossip and speculation on the conference circuit, any one of a dozen laboratories could have found it first.

For humans there was no theta antigen. But the remarkable property of human T cells forming rosettes with sheep red blood cells (Lay *et al.*, 1971) formed a surrogate assay until monoclonal antibodies were developed. There then developed a fashion for rosetting that has now passed. The most useful discovery was the property of CLL cells of forming rosettes with mouse red blood cells (Stathopoulos & Elliott, 1974).

The immunophenotype of CLL was quickly defined. As well as IgM, most cells also carried IgD (Fu *et al.*, 1974; Preud'homme *et al.*, 1974). Surface immunoglobulin density was much lower than for normal B cells (Chen & Heller, 1978). Paradoxically, an antigen initially regarded as T-cell specific and later designated CD5 was recognized on the surface of CLL cells by the monoclonal antibodies RFT-1, Leu-1 and OKT-1 (Caligaris-Cappio *et al.*, 1982).

Clinical staging

At the same time as the immunophenotype was being defined, two clinical staging systems for CLL were being developed. In New York, Kanti Rai defined five groups and gave them Roman numerals (Rai *et al.*, 1975), while in Paris Jacques-Louis Binet designated three groups alphabetically (Binet *et al.*, 1977). In reality, both systems were saying the same thing, namely that the more disease you had the worse the prognosis and if the bone marrow started to fail then the outlook was dire. It was no surprise to see the International Workshop on CLL (IWCLL) recommend that the two systems be amalgamated (IWCLL, 1981, 1989), although in reality the Americans continue to use Rai and the Europeans use Binet. A number of other prognostic

indicators have since been recognized, including lymphocyte doubling time (Montserrat *et al.*, 1986), bone marrow histology (Rozman *et al.*, 1984) and chromosomes (Juliusson *et al.*, 1990).

Differential diagnosis

Most of the large series of patients with CLL published before 1990 were contaminated with diseases that we would now recognize as not being CLL. Lymphosarcoma cell leukaemia was recognized early as something rather different (Isaacs, 1937), but criteria for its diagnosis varied between different institutions (Zacharski & Linman, 1960; Schwartz *et al.*, 1965). Aisenberg & Wilkes (1976) recognized a type of spill-over lymphosarcoma with sparse surface immunoglobulin, similar to CLL (well-differentiated lymphocytic lymphoma), that, by the revised European–American classification of lymphoid neoplasms (REAL), we would probably recognize as the same disease as CLL. Those tumours with denser surface immunoglobulin were clearly different, but lymphoma classification was so uncertain that we can only speculate on how different. Many will have been follicular lymphomas (Spiro *et al.*, 1975) or what we may perhaps refer to in the future as t(14;18) disease (Cleary *et al.*, 1986).

Prolymphocytic leukaemia (PLL) was recognized as a separate entity by Galton *et al.* (1974). Because prolymphocytes may accumulate in CLL, there has been confusion, but it should now be clear that CLL does not transform into PLL.

Hairy cell leukaemia was recognized in 1958, albeit under a different name (Bouroncle *et al.*, 1958). It is difficult to believe it could be confused with CLL, although perhaps more excuse could be made for confusing the hairy cell variant (Cawley *et al.*, 1980). Splenic lymphoma with villous lymphocytes (SLVL) was first recognized in 1979 (Neiman *et al.*, 1979), although again under a different alias. Since the REAL classification gained popularity, this is being recognized as a form of splenic marginal zone lymphoma. Other cases of this disease may not have very obvious villi and may constitute what has become known as CD5-negative CLL (Salomon-Nguyeu *et al.*, 1995).

Because it is CD5 positive, mantle cell lymphoma is the last of the lymphosarcoma cell leukaemias to be separated from CLL. It has emerged as a distinct entity via many different name changes, although its existence in a leukaemic phase was only lately recognized (De Oliveira *et al.*, 1989). It has dense surface immunoglobulin and lacks CD23, but most distinctive is the t(11;14) translocation (Raffeld & Jaffe, 1991). It is a matter of faith in our laboratory that no case of CLL has this translocation.

T-cell CLL was first described by Brouet *et al.* (1975). Several subtypes have now been recognized and the term is no longer used. Although T-PLL had been recognized in a number of prior publications, including some by the Royal Marsden team, the defining description was by Matutes *et al.* (1986). It is usually a very malignant disease with a characteristic karyotype. Despite its distinctive cellular morphology, it is still confused with CLL (Hoyer *et al.*, 1995). McKenna *et al.* (1977) first described large granular lymphocytic leukaemia. This condition usually has CD8-positive lymphocytes. It exists in CD3-positive and

CD3-negative forms (Loughran, 1993). Non-clonal proliferations are also seen. There remain the CD4-positive leukaemias that are either the disease associated with HTLV-1 or part of the mycosis fungoides–Sezary syndrome complex (Matutes *et al.*, 1988). The term T-cell CLL should no longer be used and therefore it is no longer necessary to call CLL ‘B-cell CLL’.

Complications

Patients with CLL seldom die because of a high white cell count, but there are plenty of other fatal characteristics of the disease.

Immunodeficiency. Before the Second World War, Wintrobe recognized that patients were particularly sensitive to infection (Wintrobe & Hasenbush, 1939) and we now know that most patients will develop low levels of immunoglobulin if cases are followed for long enough. Hypogammaglobulinaemia was first recognized as a clinical entity by Bruton (1952), when electrophoresis of serum revealed the surprising absence of the gamma fraction in an otherwise normal pattern. Immunological studies showed a complete absence of antibodies and isohaemagglutinins. Odd case reports of agammaglobulinaemia in CLL began appearing shortly afterwards (Brem & Morton, 1955; Jim & Reinhard, 1956). Jim (1957) found that 17 out of 50 patients had hypogammaglobulinaemia. In a more comprehensive study, Cone & Uhr (1964) found deficiencies in all classes of serum immunoglobulins and failure to produce an antibody response to φx174, a primary antigen, or diphtheria toxoid, a secondary antigen. There was also a failure to sensitize to dinitrofluorobenzene, although most patients did produce a delayed hypersensitivity response to recall antigens. Later studies correlated the more severe falls in serum immunoglobulins with more advanced disease (Fiddes *et al.*, 1972) and demonstrated that it was possible to generate an immune response to a new antigen (φx174) in early stage patients if sufficient inoculations were given (Hamblin *et al.*, 1975). Really profound immunodeficiency had to await the arrival of the purine analogues as a popular treatment (O’Brien *et al.*, 1993). Why the immunodeficiency of CLL is so much worse than that of other lymphoid tumours is one of the unsolved mysteries of the disease.

Autoimmunity. Winifred Ashby (1879–1975) moved from London to Chicago at the age of 14. She carried out her pioneering work into the life span of red cells at the Mayo Clinic between 1917 and 1921. Her technique (Ashby, 1919) involved the transfusion of red cells that were compatible with, but serologically distinct from, those of the recipient and then tracking their survival by differential agglutination. Berlin (1951) used this technique in nine patients with CLL. All had a shortened red cell survival, even though only one had a reticulocytosis. This was probably the first demonstration that the anaemia of CLL might be haemolytic in nature.

It was shortly after Ehrlich (Ehrlich & Morgenroth, 1901) published the concept of ‘horror autotoxicus’, the idea that the body would not make an antibody that destroyed its own tissues, that Donath & Landsteiner (1904) described an

antibody that did just that. Shortly afterwards, Fernand Widal (of typhoid fame) was probably the first to recognize acquired haemolytic anaemia with red cell agglutination (Widal *et al.*, 1908). Thirty years later, in Boston, Dameshek & Schwartz (1938) stressed the importance of 'haemolysins' in the commonest type of acquired haemolytic anaemia. It was not quite clear what these 'haemolysins' were until the development of the indirect anti-globulin test by Robin Coombs (Coombs *et al.*, 1945) and its application in haemolytic anaemia (Boorman *et al.*, 1946). Wasserman (not of syphilis fame) found haemolytic anaemia to be present in nine out of 58 consecutive patients with CLL; five out of seven patients tested had a positive Coombs' test (Wasserman *et al.*, 1955). A series of studies suggested that autoimmune haemolytic anaemia (AIHA) occurs at some time in the course of CLL in between 10% and 26% of cases (Wasserman *et al.*, 1955; Pisciotta & Hirschboeck, 1957; Beickert, 1959; Dameshek & Schwartz, 1959; Troup *et al.*, 1960; Videbæk, 1962).

It is often forgotten that the 'I' in ITP (immune thrombocytopenic purpura) originally stood for 'idiopathic' and not 'immune'. William Dameshek (Fig 6), although a giant of haematology almost without equal, was clearly wrong in his championing of 'hypersplenism' as the cause of thrombocytopenia. The spleen was supposed to produce a sort of miasma that inhibited the bone marrow. Harrington *et al.* (1951) first demonstrated that the plasma of patients with chronic ITP transfused into a normal recipient (himself) would produce thrombocytopenia. (Oh what experiments you could do in the world before viruses!) Later, Shulman *et al.* (1965) showed that this plasma factor was present in the 7S gamma globulin fraction and was absorbed by human platelets.

Thrombocytopenia is quite common in CLL. Minot and



Fig 6. William Dameshek, 1900–1969. The greatest haematologist of the twentieth century? Picture from US National Library of Medicine.

Buckman (1925) found it in half their patients at presentation and in virtually all those patients whose white cell count rose above $175 \times 10^9/l$. Harrington & Arimura (1961) reported seven cases of autoimmune thrombocytopenia occurring in CLL. Dameshek reported five more (Ebbe *et al.*, 1962), but because of the unsatisfactory nature of platelet antibody tests the true prevalence of ITP in CLL is unknown. An increase in bone marrow megakaryocytes remains the surest touchstone.

Reporting autoimmunity in CLL was then a popular sport. Immune neutropenia (Killman, 1959), pure red cell aplasia (Abeloff & Waterbury, 1974), Sjögren's syndrome (Lehner-Netsch *et al.*, 1969), nephrotic syndrome (Dathan *et al.*, 1974), bullous pemphigoid (Cuni *et al.*, 1974), Graves's disease (Haubenstock & Zalusky, 1985), systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, allergic vasculitis and pernicious anaemia (Miller, 1962; Dameshek, 1967) have all been associated with CLL.

The fact that CD5-positive B cells secrete antibodies that can be made to react with DNA and IgG (Stoeger *et al.*, 1989) has encouraged speculation concerning the origin of autoimmune disease in CLL. In fact, the clinically important autoantibodies are produced by the residual normal immune system, not by the tumour cells. Most of the associations between CLL and autoimmune diseases occur by chance. Only AIHA and ITP are more common than in an age-matched control population (Hamblin *et al.*, 1986). The high incidence of AIHA in fludarabine-treated patients (Myint *et al.*, 1995) suggests that autoimmunity is a consequence of the severe immunodeficiency that occurs in CLL and especially the AIDS-like syndrome that may follow treatment with fludarabine.

Transformation. Richter (1928) described an aggressive lymphoma occurring in a patient with CLL and gave his name to a phenomenon that occurs in up to 3% of patients. Histologically, the tumour is a diffuse large-cell lymphoma (Trump *et al.*, 1980). Modern techniques have demonstrated that in roughly half the cases the second lymphoid tumour is clonally unrelated to the first (Miyamura *et al.*, 1990; Kruger *et al.*, 1993). In prolymphocytic transformation of CLL (Enno *et al.*, 1979), the cell markers remain CLL like. Although of grave consequence to the patient, he does not develop PLL. Transformation to acute lymphoblastic leukaemia has been reported (Brouet *et al.*, 1973). Such was the confusion at the time over what CLL was, I doubt whether this or any of the subsequent cases were correctly assigned.

Up-to-date

Today, we think of diseases in terms of molecules. Our molecular understanding of CLL is incomplete. It had a poor start. It was not until 1979 that the first consistent chromosomal abnormality (trisomy 12) was reported (Gahrton *et al.*, 1979). We still do not know what it means. The translocations $t(11;14)(q13;q32)$ and $t(14;18)(q32;q21)$ involving the supposed B-cell leukaemia oncogenes *BCL-1* and *BCL-2* proved to relate mainly to what the ancients called lymphosarcoma cell leukaemia. Translocations at $t(14;19)(q32;q13)$ involving *BCL-3* (Ueshima *et al.*, 1985) do occur in CLL, but are vanishingly rare. The commonest

abnormality involves deletions at 13q14 (Fitchett *et al.*, 1987) and its unravelling has thrown up candidate genes that might be responsible for most cases (Liu *et al.*, 1997). An important subset of more malignant cases and bulky lymphadenopathy has deletions at 11q23 (Dohner *et al.*, 1997). As with most tumours, p53 is involved somewhere (Lens *et al.*, 1997) and not to the advantage of the patient.

A new and important cell marker, CD79b, is surprisingly absent, although probably present as a short splice variant (Alfarano *et al.*, 1999). This has been included in a cell marker definition of the disease that is remarkably helpful (Moreau *et al.*, 1997).

We no longer consider CLL to be a disease of accumulation of long-lived functionally incompetent lymphocytes. We prefer to describe the cells as anergic, activated and antiapoptotic (Caligaris-Cappio, 1996; Caligaris-Cappio & Hamblin, 1999), which comes to much the same thing. In another respect, David Galton was right. He described two types of CLL, one progressive and one stable. This can also be translated into molecular parlance. By studying the immunoglobulin variable (V) region genes, we can discern the same two distinct types. One type, derived from a naïve cell (recognized by having unmutated V genes), is progressive with a median survival of 8 years. The other type, derived from a memory cell (recognized by having mutated V genes), is stable with a median survival of 25 years (Damle *et al.*, 1999; Hamblin *et al.*, 1999).

What is remarkable about the study of CLL is how often the great doctors of the past have been scintillatingly right. What is comforting is how often they have been spectacularly wrong.

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1034 Historical Review

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