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Evolution of a terminal deoxynucleotidyl transferase-positive lymphoma from a chronic T cell lymphocytosis

D. G. Oscier[1], G. J. Mufti[1], T. J. Hamblin[1], D. B. Jones[2] & J. L. Smith[3]

[1]Royal Victoria Hospital, Bournemouth, [2]University Department of Pathology, Southampton General Hospital and [3]Regional Immunology Laboratory, Tenovus Research Institute, Southampton General Hospital, Southampton, U.K.

A 56-yr-old Caucasian man presented with a generalised scaly rash and a peripheral blood lymphocytosis of 5.6 × 10^9/l. 5 yr later he developed cutaneous nodules, lymphadenopathy and hepatosplenomegaly. Cells with convoluted nuclei and prominent nucleoli were seen in the peripheral blood. He underwent splenectomy and received intensive chemotherapy but died 6 months later with CNS infiltration. At presentation the peripheral blood lymphocytes were E-ve, UCHT1 + ve, and OKT8 + ve. Following transformation, cells in blood, spleen and CSF were E-ve, OKT11 + ve, DR + ve and Tdt + ve. A proportion of these cells had a Sézary-like appearance at E/M. The splenic cells showed functional suppressor activity. This is the first reported case of the evolution of a Tdt + ve lymphoma from a post-thymic T cell lymphocytosis.

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The normal development of T lymphocytes begins with the migration of bone marrow prothymocytes to the thymus in which a number of stages of differentiation occur before subsets of mature T cells are released into the blood and peripheral lymphoid tissue (1). The stages of differentiation have been made clearer by the application of panels of monoclonal anti-T cell antibodies together with a rabbit polyclonal antibody to the nuclear enzyme, terminal deoxynucleotidyl transferase (Tdt), which is found in prethymic and thymic, but not post-thymic, T cells.

T cell malignancies have been classified into a number of distinct entities according to clinical, morphological, cytochemical, histological, karyotypic, functional, virological and immunological criteria (2, 3). It is well-recognised that T cell malignancies of mature or post-thymic T cells may undergo clinical and morphological tranformation to a more aggressive disease, but where immunological phenotypic analysis has been performed the cells retain the post-thymic phenotype. We report a multiparameter study of a patient with a T cell malignancy who presented initially with the features of a mature post-

thymic tumour which subsequently evolved into a Tdt-positive lymphoma.

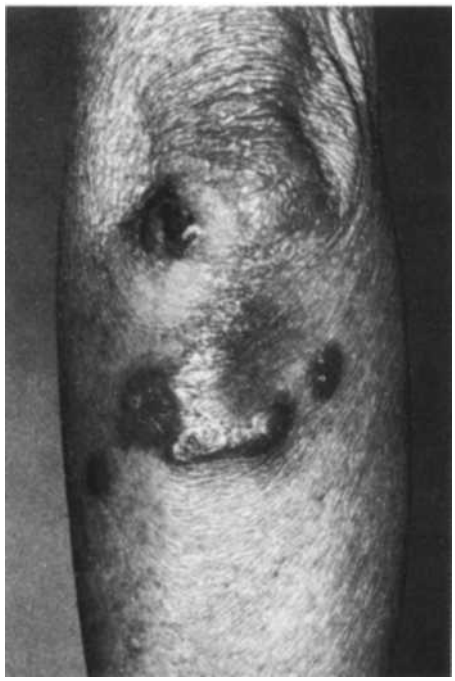

Figure 1. Nodules on elbow.

Methods

Lymphocyte markers

Lymphocytes from peripheral blood were prepared by Ficoll-Triosil separation, the cell layer being resuspended after washing in bovine serum albumin - minimum essential medium (BSA-MEM) at a concentration of 2×10^6/ml. Lymphocytes from cerebrospinal fluid were washed 3 times and then resuspended in BSA-MEM at a concentration of 2×10^6/ml. Spleen cells were prepared by mincing the tissue and then extruding through sterile wire mesh before Ficoll-Triosil separation, washing and resuspending in BSA-MEM at a final concentration of 2×10^6/ml.

Rosette formation was used to examine for the presence of sheep red cell receptors and Fcγ receptors. Sheep red blood cells were obtained from an animal known to give consistent results in our laboratory and were used after washing without further treatment. For Fcγ receptors, ox red blood cells coated with IgG from a rabbit anti-ox red cell antiserum were used as the indicator. Other cell markers were detected by immunofluorescence. Immunoglobulin heavy and light chains were examined using direct immunofluorescence with fluoroscein-labelled sheep antibodies (Tenovus Institute). Indirect immunofluorescence using fluoroscein-labelled antibody to mouse immunoglobulin (DAKO immunoglobulins) was used for the following mouse monoclonal antibodies:- OKT4, OKT8, OKT6, OKT10, OKT11 (Ortho Pharmaceuticals), UCHT1 (a gift from Peter Beverley), FMC4 (Seralab), J5 (Coulter Electronics) and Leu7 (Becton Dickenson). Tdt activity was detected by indirect cytoplasmic immunofluorescence on methanol-fixed cells using rabbit polyclonal antibody to calf thymus Tdt with fluoroscein-labelled goat anti-rabbit serum (Bethesda Research Laboratories).

Assay of suppressor activity (5)

A suspension of spleen cells was prepared by thoroughly mincing and extruding the splenic tissue through sterile wire mesh, followed by Ficoll-Triosil separation. After 3 washes in Ca^{++}, Mg^{++} free Hanks Balanced Salt Solution (CM-F · HBSS) patient's cells were cultured at 1×10^6/ml in 5 ml cultures in RPMI 1640 with 10% fetal calf serum added. Cells were cultured for 24 h at 37°C in 5% CO_2/air in the presence or absence of concanavalin A at 10 μg/ml[12] (Con A, Miles Laboratories, Batch No. 180, Cat 79-003). After the initial culture period, cells were washed a further 3 times in CM-F · HBSS, adjusted to 1×10^6/ml and both Con A-stimulated and control cells were added to normal responder mononuclear cells in

TABLE 1

Figures represent percentage of cells expressing each marker. (Spleen cells were unreactive with OKT6, OKT10, Leu7 and J5)

Tissue	Date	SmIg	Fc	E	TII	UCHT1	T4	T8	DR	TdT
Blood	14. 2.79	4	37	24						
Blood	23. 6.81	8	54	18		80	4	50		0
Blood	25.11.81	12		5					60	12
Spleen	23. 3.82			2	90	30	14	14	85	85
CSF	19. 8.82				100					100

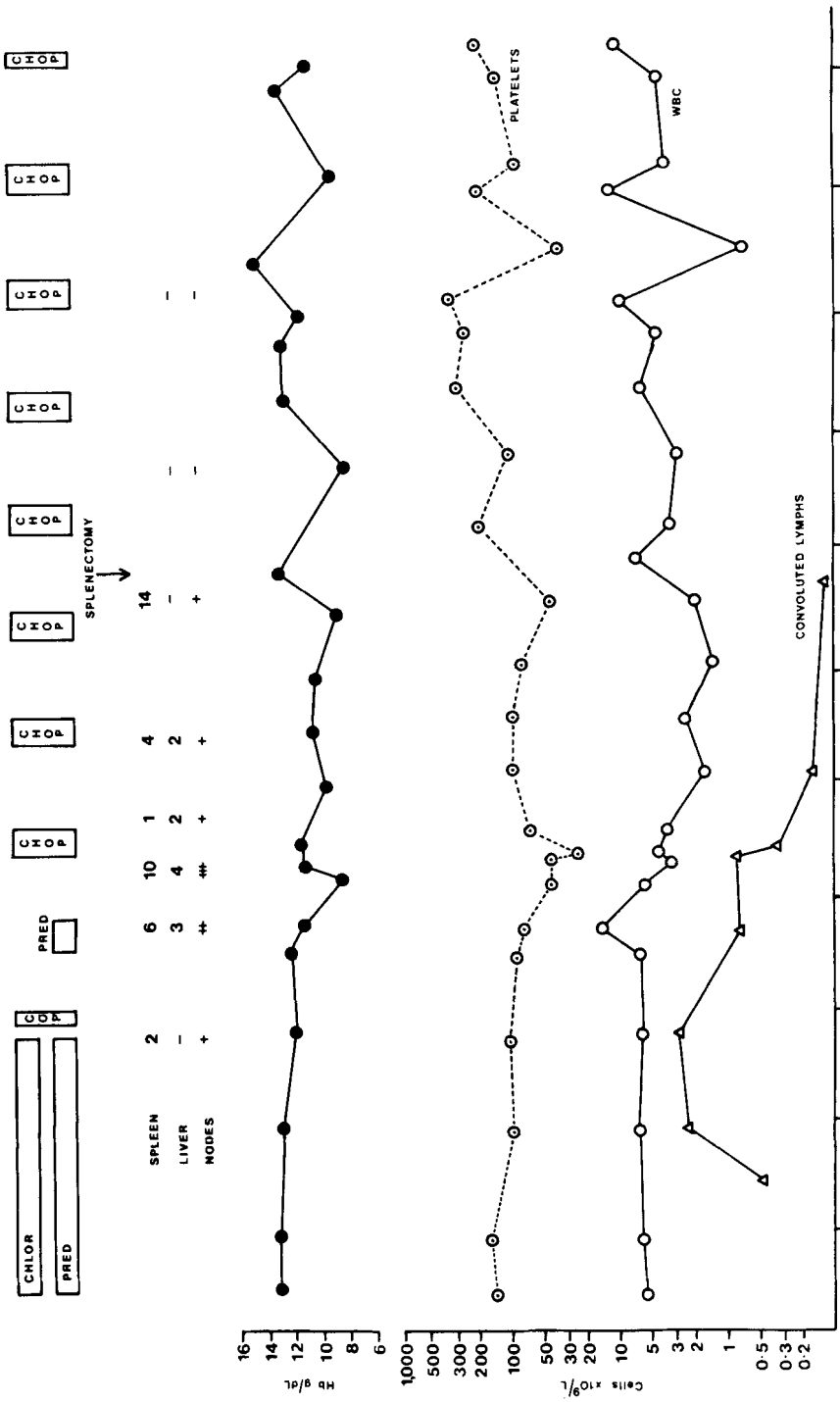

Figure 2. Summary of the haematological data and the treatment received.

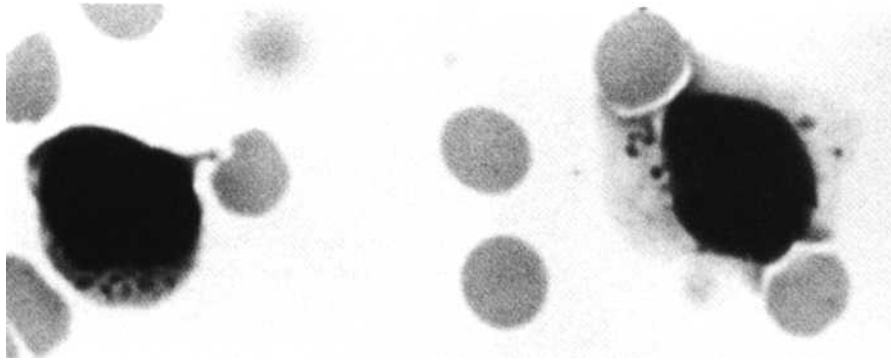

Figure 3. Peripheral blood showing lymphocytes with azurophilic granules.

microwell plates. The ratio of control or mitogen-stimulated patient's cells to the responding population was 1:3. These cell mixtures were cultured for a further 48 h. Suppression was calculated as the reduction in responder cell proliferation to Con A in the presence of activated patient's cells over that observed with responder cells cultured alone or with unstimulated patient's cells. Proliferation data were obtained from the mean ^{3}H incorporation in 6 wells for each cell combination.

Case history and results

A 56-yr-old Caucasian male (SN) presented in January 1979 with a 3-yr history of a generalised scaly rash. On examination he had small (< 1 cm) cervical, axillary and inguinal lymph nodes but no hepatosplenomegaly. A skin biopsy showed unspecific inflammatory changes in the dermis. A full blood count revealed an Hb of 126 g/l, a WBC of 13.6 $\times$ 10^9/l with 51% neutrophils and 41% lymphocytes, and a platelet count of 214 $\times$ 10^9/l. The majority of the lymphocytes had azurophilic granules in the cytoplasm (Figure 3) and lymphocyte marker studies gave the following results: -E 24%, Fcγ 37%, SIg 4% (Table 1). S-immunoglobulins, S-calcium and a chest X-ray were normal. He was treated unsuccessfully with short courses of steroid creams, synacthen depot injections and oral methotrexate 12.5 mg wk.

His condition remained stable until June 1981 when he developed indurated nodular lesions on the elbows and knees (Figure 1). A biopsy of 1 of these nodules revealed a sparse perivascular infiltration of lymphoid cells with convoluted nuclei and showing epidermatropism. Electron microscopy showed small numbers of cells with cerebriform nuclei. Immunostaining of frozen tissue demonstrated that the majority of lymphocytes were T4-ve T8+ve. The peripheral blood WBC was 5.8 $\times$ 10^9/l with 60% lymphocytes which had the following marker profile: -E 5%, UCHT1 80%, T8 50%, T4 4%, SmIg 12%, Tdt-negative. 80% of lymphocytes showed multiple granular cytoplasmic staining with acid phosphatase and unspecific esterase.

The subsequent clinical course is shown in Figure 2. In October 1981 he was treated with chlorambucil 10 mg/d and prednisolone 10 mg/d. After 2 months there had been slight improvement of the skin lesions but the supraclavicular and axillary lymph nodes had enlarged and the spleen was now palpable 2 cm below the left costal margin. The WBC was 6.3 $\times$ 10^9/l with 60% lymphocytes. Examination of the peripheral blood film now showed a population of large cells with convoluted nuclei (Figure 4) and another population of cells with a deeply basophilic cytoplasm and a primitive nucleus with prominent nucleoli (Figure 5). These cells stained weakly with UCHT1 and OKT8 but were strongly DR+ve and 12% were Tdt+ve. A bone marrow aspirate was hypercellular and contained 16% abnormal lymphoid cells. A marrow trephine showed both focal and diffuse involvement with similar cells. Chromosomal analysis of the bone marrow cells showed the presence of 2 distinct cell lines. There was a diploid clone with an apparently normal male karyotype and a hyperdiploid clone with 83 chromosomes. A lymphangiogram showed filling defects in the para-aortic and inguinal nodes consistent with lymphomatous involvement. His treatment was changed to cyclophosphamide, vincristine and prednisolone (COP), but after 1 course there was a further increase in lymphadenopathy and his liver was palpable 4 cm and spleen 10 cm

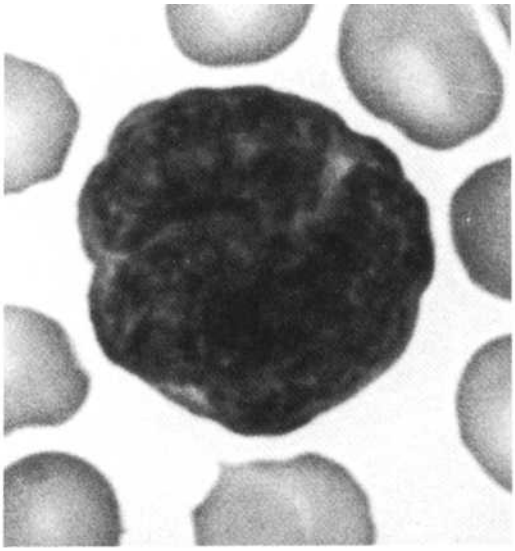

Figure 4. Peripheral blood showing a lymphocyte with convoluted nuclei.

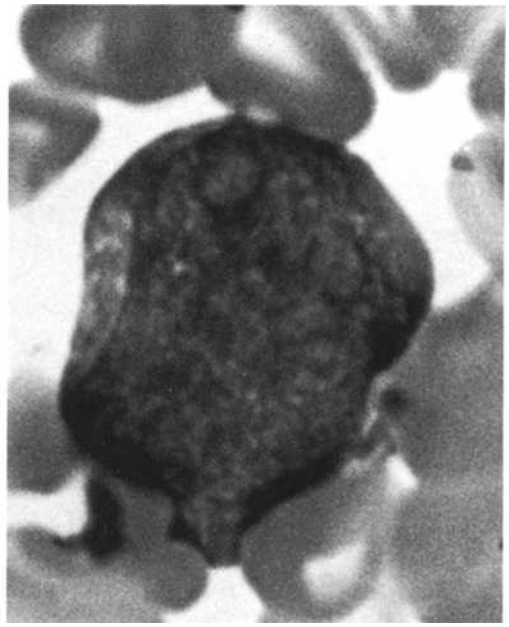

Figure 5. Peripheral blood showing a blast cell with prominent nucleoli.

below their respective costal margins. He was then treated with cyclophosphamide, adriamycin, vincristine and prednisolone (CHOP) and by March 1982, after 2 courses, there was complete regression of the skin nodules, lymph nodes and hepatosplenomegaly. However, 3 wk later his spleen was 14 cm enlarged and small inguinal and axillary nodes were palpable. A full blood count showed an Hb of 8.7 g/l, WBC count of 2 $\times$ 10^9/l with 4% abnormal lymphoid cells and a platelet count of 44 $\times$ 10^9/l. A marrow aspirate was normocellular and contained 3% abnormal lymphoid cells. A splenectomy was performed and the spleen weighed 3.4 kg. There was destruction of the normal splenic architecture with infiltrating sheets of large irregular lymphoid cells. Electron microscopy revealed 2 major populations of cells, those with irregular nuclei and a single large nucleolus (Figure 6) and those with highly convoluted nuclei (Figure 7). The phenotype of the splenic cells was T11+ve, DR+ve, Tdt+ve, T3-ve, T6-ve, T9-ve, T10-ve, J5-ve.

The results of the suppressor assay on the spleen cells are shown in Table 2. Con A-activated patient's cells suppressed Con A-induced proliferation of normal lymphocytes more than Con CHOP during which time his skin remained scaly, but he had no skin nodules, lymphadenopathy or hepatomegaly. No abnormal lymphoid cells were seen in the peripheral blood. In August 1982 he presented with multiple cranial nerve palsies, grand mal fits, weakness of the legs and a sensory level at T8. CSF examination revealed abnormal lymphoid cells which were T11+ve, Tdt+ve. No further treatment was given and he died shortly afterwards. At postmortem there was infiltration of the liver, lymph nodes, lungs, kidneys, skin, meninges, spinal cord and bone marrow with large lymphoid cells. These cells

TABLE 2
Results of suppressor cell assay

	^{3}H uptake (mean of 6)	% Reduction
Responding cells + SN	50 114	49.3%
Responding cells + Con A-activated SN	25 363	
Responding cells + con	49 047	25.8%

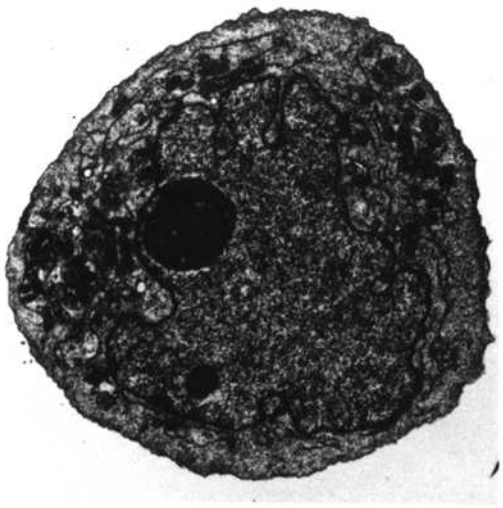

Figure 6. E/M of a spleen cell with prominent nucleolus (× 7180).

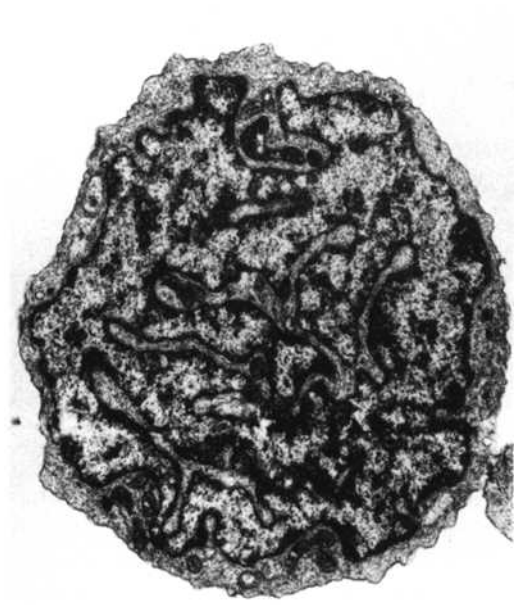

Figure 7. E/M of a spleen cell with convoluted nucleus (× 7120).

had moderately abundant cytoplasm, irregular or convoluted nuclei and a single prominent nucleolus.

Discussion

This case has a number of unusual features which do not allow it to be classified under any of the well recognised groups of T cell tumours. The initial presentation of a generalised rash with an unspecific dermal infiltrate, and a slight lymphocytosis comprising small lymphocytes with azurophilic granules and a membrane phenotype E-ve Fc γ+ve UCHT1+ve T8+ve T4-ve Tdt-ve would be compatible with a diagnosis of T-CLL (6). However, the possibility that the lymphocytes were natural killer cells was not excluded, nor was there conclusive proof that the lymphocytosis was truly malignant rather than 'reactive'. Throughout the course of the illness, lymphocytes from peripheral blood and spleen did not form rosettes with sheep red blood cells despite reacting with OKT11. This has previously been reported in T cell tumours and it is not clear whether this is due to a serum blocking factor or to differential sensitivity of the 2 tests for the same receptor (7, 8). After 2 yr the disease entered a more aggressive phase characterised by cutaneous nodules and the presence in the blood and skin of cells with cerebriform nuclei which, on electron microscopy, had many similarities to Sézary cells. However, these cells had suppresor/cytoxic phenotype, whereas classical Sézary cells have a helper phenotype and in many cases have functional helper activity (9). Kansu & Hauptman (10) reported a patient with Sézary syndrome in which the cells had suppressor activity but, unfortunately, no surface markers other than E rosettes were performed. After an initial response to chemotherapy there was rapid enlargement of the spleen due to proliferation of cells with the histological appearance of T-immunoblastic sarcoma. These cells had a phenotype which was DR-positive, Tdt-positive but T8-negative, and showed functional suppressor activity after stimulation with concanavalin A. Immunoblastic transformation of cutaneous T cell lymphomas (CTCL) has previously been

documented. Lawrence et al (11) described a case of CTCL progressing to a large cell lymphoma in which the large cells had functional helper activity. Knowles & Halper (12) reported 2 similar cases in which the helper phenotype of the original cells was retained in the immunoblasts. Yanagihara et al (13) have documeted a case of CTCL with transformation to an immunoblastic sarcoma and in which the transformed cells in blood, lymph node and skin all expressed OKT11, T4, and T8. The most interesting aspect of this case is the expression of Tdt in spleen cells and terminally in cells obtained from the CSF. These cells did not express other markers typically found in thymocytes such as OKT6 and OKT10. Tdt is a nuclear enzyme normally found only in prethymic and thymic T cells. Among the T cell tumours it is only found consistently in T-ALL and T-lymphoblastic lymphoma (14), although Knowles & Halper have documented 2 cases of multilobated T cell lymphoma (12) which were Tdt-positive and we have seen a case with the same histological appearance in which 10% of the cells were Tdt-positive. Penit et al (16) found Tdt positivity in 1 out of 3 cases of Sézary syndrome, but they measured Tdt enzymatically and this technique is now considered to be less accurate than immunocytochemical methods. There is evidence that the skin may have an effect on T cell maturation independent of the thymus. Haynes et al (17) found thymic markers on malignant T cells in the skin but not in the blood of 2 patients with CTCL. Rubenfeld et al (18) showed that human epidermal cell cultures were able to induce the expression of Tdt in a small percentage of E rosette-positive and null cells from peripheral blood and bone marrow in co-culture experiments. Since the aggressive phase of the disease manifested in the skin, such a mechanism is possible in our patient.

In summary, our patient presented with a lymphocytosis of post-thymic T cells and subsequently developed an aggressive lymphoma which was Tdt + ve and had functional suppressor activity. Such a prodromal phase is not a feature of the recognised Tdt + ve T cell tumours and, in this respect, our patient is unique.

Acknowledgement

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Correspondence to:
Dr. D. G. Oscier
Consultant Haematologist
Royal Victoria Hospital
Shelley Road
Bournemouth, BH1 4JG
U.K.