threonine phosphorylation can affect integrin adhesiveness[17], the substrates involved have not been identified[18].

A model has been proposed in which integrins are endocytosed at the rear of the cell and are recycled forward[19-21]. Many integrins, including αvβ3, have been shown to be endocytosed[22], and it has been shown that recycling receptors are preferentially inserted at the leading edge of migrating cells[23]. It has been difficult to test this model because inhibitors of constitutive endocytotic recycling are nonselective[24]. Because αvβ3 integrins on neutrophils require a specific signalling mechanism to be released from the substrate and endocytosed, we could selectively block this process.

To study endocytosis of αvβ3 integrins, we used a monoclonal antibody that binds to αv without blocking binding to vitronectin[25]. (Preincubation with this antibody does not affect the distribution of β3 integrins in the lower adherent membrane of control or $[Ca^{2+}]_i$-buffered cells.) Neutrophils were incubated in suspension with the antibody, plated on vitronectin and then stimulated to migrate. After 5 min the cells were fixed, permeabilized and labelled with a fluorescent secondary antibody. Figure 3*a*, *c* shows top and side views of a migrating neutrophil. The αv integrin is found in numerous vesicles throughout the cell. These vesicles may correspond to the specific granules that were shown previously to contain a vitronectin receptor[26]. Because the primary antibody was initially bound to the surface of intact cells, these integrins must have been endocytosed into the cell.

When $[Ca^{2+}]_i$ was buffered, the integrins were found clustered toward the rear of the cell on the lower surface (Fig. 3*b*, *d*). The low level of intracellular staining in the $[Ca^{2+}]_i$-buffered cells shows that essentially all of the recycling integrins had become trapped on the lower surface during the 5 min that the cells were attached to vitronectin. In separate experiments (not shown), β3 integrins were localized in control or $[Ca^{2+}]_i$-buffered cells by fixing the cells, permeabilizing them and then labelling with antibodies to β3 cytoplasmic domains and fluorescent secondary antibodies. The distribution of the total β3 pool was similar in both cases to the distribution of labelled αv shown in Fig. 3. This indicates that essentially all of the αvβ3 integrins are cycling between the surface and endosomes and that they become trapped on the adherent membrane when $[Ca^{2+}]_i$ is buffered.

As shown schematically in Fig. 4, preferential insertion of αvβ3 integrins at the leading edge along with $[Ca^{2+}]_i$-regulated detachment and clearance from the adherent cell surface provides a mechanism for creating a gradient of adhesive strength from the front to the rear of a migrating cell. The inability of neutrophils to migrate on adhesive substrates when this process is blocked provides a clear demonstration that in these cells recycling of integrins to the front of the cell is required for continued migration on vitronectin. □

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Mortality before and after HIV infection in the complete UK population of haemophiliacs

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DURING 1977–91, 6,278 males diagnosed with haemophilia were living in the UK. During 1979–86, 1,227 were infected with the human immunodeficiency virus (HIV-1) as a result of transfusion therapy (median estimated seroconversion date, October 1982). Among 2,448 with severe haemophilia, the annual death rate was stable at 8 per 1,000 during 1977–84; during 1985–92 death rates remained at 8 per 1,000 among HIV-seronegative patients but rose steeply in seropositive patients, reaching 81 per 1,000 in 1991–92. Among 3,830 with mild or moderate haemophilia, the pattern was similar, with an initial death rate of 4 per 1,000 in 1977–84, rising to 85 per 1,000 in 1991–92 in seropositive patients. During 1985–92, there were 403 deaths in HIV seropositive patients, whereas 60 would have been predicted from rates in seronegatives, suggesting that 85% of the deaths in seropositive patients were due to HIV infection. Most of the excess deaths were certified as due to AIDS or to conditions recognized as being associated with AIDS.

Since 1976 the UK National Haemophilia Register[1] has included all UK residents diagnosed with haemophilia A (classical haemophilia, factor VIII deficiency) or haemophilia B (Christmas disease, factor IX deficiency). During 1977–91, 2,448 males with severe haemophilia, and 3,830 males with moderate or mild haemophilia were included in the Register and, on 1 January 1993, 82% were alive, 15% had died and 3% were lost to follow-up (Table 1).

During 1979–86, blood products used to treat haemophilia carried a risk of HIV-1 infection, and 4,043 patients (2,037 severe, 2,006 moderate or mild) are recorded as having received

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TABLE 1 Males included in the UK National Haemophilia Register, 1977–91, by severity of haemophilia, HIV-test status and vital status

Vital status on 1 January 1993	Severe haemophiliacs* Tested seropositive for HIV		Moderate or mild haemophiliacs† Tested seropositive for HIV		All patients
	No	Yes	No	Yes	
Alive and living in the UK	1,195 (84%)	673 (66%)	3,132 (86%)	135 (65%)	5,135 (82%)
Dead	198 (14%)	341 (33%)	326 (9%)	72 (35%)	937 (15%)
Emigrated	2 (0.1%)	6 (0.6%)	16 (0.4%)	0 (—)	24 (0.4%)
Lost to follow-up	33 (2%)	0 (—)	149 (4%)	0 (—)	182 (3%)
Total	1,428 (100%)	1,020 (100%)	3,623 (100%)	207 (100%)	6,278 (100%)

Several smaller haemophilia cohorts from the UK reported previously are included in the present study[16–19,23,24]. Vital status was ascertained from individual Haemophilia Centres and the National Health Service Central Registers. For each person a 'date last seen' was established. For those lost to follow-up this was the date of last contact with a Haemophilia Centre, whereas for other patients it was the earliest of: date of death, date of emigration, or 1 January 1993. HIV test results were collected in a series of annual surveys starting in 1985 (ref. 3). For 441 patients who were tested and found to be seropositive, the results of a previous seronegative test were available. Patients diagnosed or treated in the UK but living abroad are excluded, as are the few female patients. In addition, two severely affected patients (including one who had been tested seropositive for HIV) and two moderately affected patients, whose years of registration were after they had died, were excluded.

* Factor VIII or IX level of less than two international units per dl.

† Includes 104 patients with unknown severity, two of whom were tested seropositive for HIV.

potentially infected treatments. A reliable test for HIV antibodies[2] became available to Haemophilia Centres early in 1985. Among those who were alive on 1 January 1985, 78% of potentially infected severe patients and 52% of moderate/mild patients had been tested by December 1985, rising to 90 and 74% respectively by January 1993. One thousand and twenty severe patients and 207 moderate/mild patients were found to be infected with HIV (described as tested seropositive) (Table 1). For many patients, stored serum samples enabled the seroconversion date to be estimated reasonably precisely[3–5]. The median estimated date of seroconversion was October 1982 for severe patients (range, June 1979–October 1986) and December 1982 for moderate/mild patients (range, October 1979–March 1986).

The annual death rate in patients with severe haemophilia remained steady at 8 per 1,000 during 1977–84, but then rose progressively to 38 in 1991–92 (Fig. 1*a*). This increase was confined to patients who tested seropositive for HIV and among whom the death rate increased steeply from 1985, reaching 81 in 1991–92; but in patients not tested as seropositive, the death rate during 1985–92 was 8 per 1,000, much as during 1977–84 (Fig. 1*c*). Among moderate/mild patients, the death rate during 1985–92 was 5 per 1,000, much as its value of 4 per 1,000 during 1977–84 (Fig. 1*b*). However, when HIV-seropositive patients were considered separately, the death rate again rose steeply during 1985–92 (Fig. 1*d*). Death rates during 1985–92 for patients tested for HIV and found not to be infected (tested seronegative) were close to rates for patients of unknown HIV status within each severity group (Table 2). Thus little, if any, HIV-associated mortality has gone undetected.

The severely affected haemophiliacs had a higher initial mortality rate and also received much more transfusion therapy than patients with moderate/mild haemophilia, yet the excess death rate associated with HIV seropositivity was similar in patients with severe and with mild/moderate haemophilia (Table 2). In both groups excess mortality associated with HIV seropositivity increased progressively with time, the rates being 19, 34, 53 and 76 per 1,000 in the periods 1985–86, 1987–88, 1989–90 and 1991–92, respectively, for both groups combined (95% confidence intervals (CIs): 13–26, 26–42, 43–63, 63–89). Treatment, by prophylaxis against *Pneumocystis carinii* pneumonia[6] or with zidovudine[7,22], has been widespread for HIV-infected haemophiliacs since about 1989. However, the steady increase in the excess death rate from 1985 to 1992 suggests that in this population the increasing impact of HIV-associated mortality has not been halted by these treatments. This study includes deaths only to 1992, and so does not permit examination of data following widespread use in the UK of high purity factor concentrate[8].

Use of the certified cause of death allows comparison of mortality rates from specific causes with those for the nation as a whole. Among patients with severe haemophilia who were not tested seropositive for HIV, there were significant increases in mortality during 1985–92 from coagulation defects, intracranial haemorrhage, injury, poisoning and suicide, and from hepatitis, liver disease and primary liver cancer, which are associated with chronic hepatic infections (Table 3). For all these causes com-

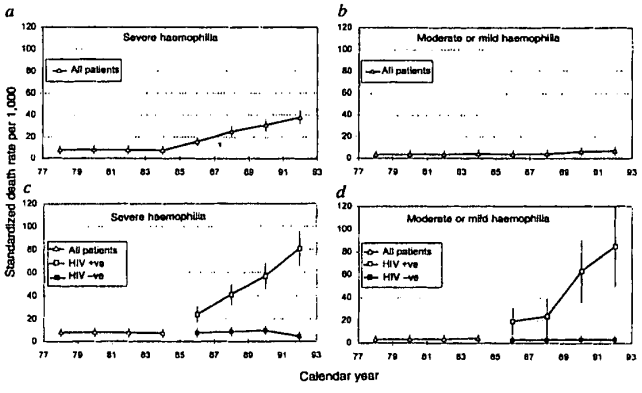

FIG. 1 Annual death rates per 1000, directly standardized for age, and 95% confidence intervals, by calendar year and severity of haemophilia. Panels a and b give values for all patients in each severity group. Panels c and d give separate values for HIV seropositive patients and patients not known to be HIV seropositive from 1985. Rates were obtained by calculating death rates (ratio of observed deaths to person-years at risk) in age-groups <15, 15–24, 25–34, 35–44, 45–54, 55–64, 65–84 by calendar period (1977–78, 1979–80, . . . , 1991–92). Person-years at risk were calculated by considering the length of time from registration to the date last seen (see Table 1) for each patient. Observed deaths and person-years over age 84 were excluded. After early 1985, patients becoming ill are likely to have been tested. Therefore, here and in Tables 2 and 3, patients tested seropositive for HIV with estimated seroconversion dates before 1 January 1985 are counted as seropositive from 1 January 1985, while the 93 patients with estimated seroconversion dates on or after 1 January 1985 contribute to the group of those not tested seropositive until their date of seroconversion, estimated as in ref. 4. For age-standardization, a weighted average of the age-specific death rates was calculated, with weights proportional to the total number of person-years at risk in the HIV seropositive patients in the whole period 1985–92 for both severity groups combined. Confidence intervals were calculated using the normal approximation.

ıs, annual death rates per 1,000, and annual excess death rates per 1,000 in HIV-seropositive haemophiliacs, by severity of haemophilia and calendar period

	Patients with severe haemophilia								
	ɜverely patients		HIV status						Excess death rate in seropositive patients*
			Unknown		Seronegative		Seropositive		
	O§	Death rate	O	Death rate	O	Death rate	O	Death rate	
1977–78	25	7.9	25	—	0	—	0	—	—
1979–80	30	8.1	30	—	0	—	0	—	—
1981–82	31	7.9	31	—	0	—	0	—	—
1983–84	30	7.5	23	—	0	—	7†	—	—
1977–84	116	7.9 (6.4–9.4)‡	—	—	—	—	—	—	—
1985–86	66	15.6	15	6.2	8	15.0	43	23.9	16.2
1987–88	98	25.0	11	8.2	13	9.3	74	41.3	33.6
1989–90	118	30.7	9	10.0	13	9.9	96	56.8	49.2
1991–92	136	37.9	8	6.1	7	3.6	121	80.8	73.2
1985–92	418	27.1 (24.4–29.8)	43	7.3 (4.7–9.8)	41	8.1 (5.4–10.9)	334	49.1 (43.7–54.4)	41.4 (35.8–47.0)

	Patients with moderate or mild haemophilia								
	All moderately or mildly affected patients		HIV status						Excess death rate in seropositive patients*
			Unknown		Seronegative		Seropositive		
	O	Death rate	O	Death rate	O	Death rate	O	Death rate	
1977–78	21	3.4	21	—	0	—	0	—	—
1979–80	28	3.5	28	—	0	—	0	—	—
1981–82	33	3.6	33	—	0	—	0	—	—
1983–84	50	4.2	46	—	1†	—	3†	—	—
1977–84	132	3.7 (3.0–4.5)	—	—	—	—	—	—	—
1985–86	49	3.8	31	2.8	5	2.4	13	19.4	16.3
1987–88	46	4.1	22	3.4	14	2.0	10	23.8	20.6
1989–90	69	6.5	28	2.4	19	4.6	22	63.0	59.9
1991–92	78	6.9	28	2.8	26	4.1	24	84.7	81.6
1985–92	242	5.4 (4.5–6.3)	109	2.9 (2.2–3.6)	64	3.5 (2.3–4.6)	69	45.2 (33.7–56.7)	42.1 (30.6–53.6)

Death rates calculated and age-standardized and confidence intervals calculated as for Fig. 1. Observed deaths and person-years over age 84 were excluded. Separate death rates for: (1) patients tested seronegative for HIV, and (2) patients of unknown HIV status, calculated by subdividing observed deaths and person-years during 1985–92 among those not tested seropositive for HIV into those who tested seronegative, with no potentially infected treatments recorded during the same or a subsequent calendar year, and others.

* Excess death rates obtained by subtracting from each calendar period-, age- and severity-specific death rate for HIV-seropositive patients, the corresponding age- and severity-specific rate for seronegative patients and patients of unknown serostatus combined, 1985–92. Age standardization and confidence intervals for excess death rates calculated as in Fig. 1.

† HIV testing was not generally available before 1985. Although some patients dying before this were tested, testing was not carried out retrospectively for the majority of patients who died. Therefore death rates by HIV status cannot be calculated before 1985. The certified causes of death of the 10 known seropositive patients dying in 1983–84 were: haemophilia (2), suicide (2), cerebrovascular accident, cirrhosis, coronary thrombosis, diabetes mellitus, myocardial infarction, renal failure. None suggests immunodeficiency. Before 1985 only one death, in a patient not reported as tested for HIV by any Haemophilia Centre, was certified as due to AIDS.

‡ 95% confidence intervals in parentheses.

§ O, observed deaths.

bined (category B) the ratio of observed to national expected deaths (O/E) was 13.3 (95% CI 10.0–17.2). Most of these associations have been reported elsewhere for haemophiliacs[9,15]. Ischaemic heart disease mortality was lower than expected, as in other haemophilia populations[11]. For other causes, mortality was similar to that in the general population ($O/E = 1.1$, 95% CI 0.7–1.7, category D). Patterns of cause-specific mortality for all patients with severe haemophilia during 1977–84 were similar (data not shown).

During 1985–92, 403 deaths occurred in seropositive patients and for 235 of these the certified cause was AIDS (ICD-9 code 279.1; Table 3). For the remaining 168 deaths in HIV-seropositives, there were significant excesses for many causes indicative of AIDS, including infections, non-Hodgkin's lymphoma and pneumonia, and also significant excesses for causes associated with haemophilia. Information received from the Haemophilia Centres indicates that many of these patients had in fact developed AIDS, indicating that in AIDS patients there is a tendency to attribute cause of death to diseases associated with haemophilia or AIDS rather than to AIDS itself. However, not all the excess mortality in patients tested seropositive for HIV appears to be due to recognized AIDS indicator diseases, and some may be due to other conditions such as liver disease.

The UK National Haemophilia Register data provide a unique opportunity to examine the impact of HIV-1 infection in a complete population where almost all potentially infected individuals have been tested. These are the first data to document that, in a large and complete population, mortality among those who by chance were infected with HIV increased more than tenfold while remaining unchanged over time in those who escaped infection (Fig. 1*c*, *d* and Table 2). Assuming that the

TABLE 3 Cause-specific mortality during 1985–92 by HIV status compared with national mortality

Certified cause of death (ICD-9 codes)	Tested seropositive for HIV: No* O‡	E§	O/E	Yes† O	E	O/E
(A) AIDS, HIV, etc. (279.1)	0	0.10	0.0	235	0.12	1,958.3***
(B) Causes significantly increased in severe haemophilia without HIV						
Hepatitis and liver disease (070, 570–573)	6	0.37	16.2***	11	0.30	37.0***
Liver cancer (155.0–155.1)	2	0.11	18.7*	1	0.07	15.1
Coagulation defects, etc. (280–289)	33	0.11	307.2***	72	0.06	1,155.7***
Intracranial haemorrhage (ICH, 430–432)	5	0.49	10.2***	1	0.37	2.7
Injury, poisoning and suicide (E800–999)	10	3.14	3.2**	8	3.68	2.2
All causes in category (B)	56	4.21	13.3***	93	4.47	20.8***
(C) Ischaemic heart disease (IHD, 410–414)	5	10.37	0.5	5	5.74	0.9
(D) Other causes						
Infections excl. hepatitis (001–139, excl. 070)	0	0.23	0.0	11‖	0.14	75.6***
Hodgkin's disease (201)	0	0.06	0.0	2¶	0.07	29.1**
Non-Hodgkin's lymphoma (200, 202)	0	0.29	0.0	12	0.21	57.1***
Other neoplasms excl. liver (140–239 excl. 155.0–.1 and 200–2)	9	9.38	1.0	7#	5.28	1.3
Endocrine disorders excl. AIDS, HIV, etc. (240–279 excl. 279.1)	1	0.50	2.0	1	0.30	3.3
Mental disorders (290–319)	0	0.35	0.0	2	0.25	8.1
Nervous system incl. dementia (320–389)	1	0.78	1.3	6	0.54	11.2***
Circulatory excl. IHD and ICH (390–459 excl. 410–4 and 430–2)	7	3.86	1.8	6	1.85	3.2*
Pneumonia (480–486)	0	0.63	0.0	12	0.31	38.6***
Other respiratory (rest of 460–519)	2	2.14	0.9	2	0.99	2.0
Digestive system excl. liver (520–579 excl. 570–573)	0	0.63	0.0	3	0.34	8.7*
Musculoskeletal and connective tissue (710–739)	1	0.11	9.0	2	0.06	33.8*
Other diseases (580–709, 740–799)	1	1.03	1.0	2☆	0.36	5.5
All causes in category (D)	22	20.00	1.1	68	10.70	6.4***
(E) Death certificate not located	1	—	—	2	—	—
All causes	84	34.68	2.4***	403	21.03	19.2***

Death details obtained from the Office of Population Censuses and Surveys (OPCS) or the General Register Offices (GRO) in Edinburgh or Belfast. Underlying cause coded to the ninth revision of the International Classification of Diseases (ICD-9)[20] by OPCS. The final certified cause may differ from that available to the public if the certifier indicates that further information may become available, and later supplies this confidentially. This system, little used in the 1970s, is commonly used for HIV-related disease[21]. The numbers of deaths with final certified cause in each category were obtained from OPCS and the GROs and the number of deaths certified to code 279.1 increased by 47, from 188 to 235 for patients tested seropositive for HIV and remained at zero for patients not tested seropositive. Observed and expected deaths over age 84 are excluded.

*** $P<0.001$, ** $P<0.01$, * $P<0.05$ (two-sided Poisson test).

* Patients with severe haemophilia not tested seropositive for HIV.

† Patients with severe or moderate/mild haemophilia tested seropositive for HIV. 83% of person-years are for patients with severe haemophilia.

‡ *O*, observed deaths.

§ *E*, expected deaths from national rates, calculated by multiplying the number of person-years in each calendar year and 5-year age group by the corresponding death rate for males in England and Wales.

‖ Septicaemia, viral encephalitis, herpes zoster, retrovirus infection (2), toxoplasmosis (4), *Pneumocystis* (2). All suggest immunodeficiency, except possibly septicaemia.

¶ Review of the clinical notes for one of these patients showed that he had a cerebral non-Hodgkin's lymphoma as well as Hodgkin's disease.

One case each of carcinoma of bronchus, duodenum, colon, rectum, pancreas, osterosarcoma and neurofibromatosis.

☆ For both deaths the certificate indicated that the cause was unknown.

death rate during 1985–92 among infected patients would, in the absence of HIV, have been close to that for uninfected patients, 60 deaths would have been predicted, whereas 403 deaths in fact occurred, an excess of 343. Thus 85% of the deaths in HIV seropositive patients are likely to have been caused by HIV. This large excess, together with the temporal pattern of the increase in those who became infected, the similarity of the excess death rate associated with HIV infection regardless of the severity of haemophilia, and the large increase in mortality from conditions not usually associated with haemophilia, demonstrate particularly clearly the enormity and the specificity of the effect of HIV-1 infection on mortality in this population. □

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