It is well established that both mH and MHC antigen disparities initiate GVH reactions.[1,18] Whereas the application of current serological, biochemical, and genetic techniques can detect MHC differences with increasing precision,[8,9,19] defining mH antigen differences in vitro has proved difficult, requiring T cells that have first been primed in vivo before they are restimulated in vitro. Previously these T cells have been derived from BMT recipients who were grafted with donor marrow from an HLA-identical sibling.[18] Our findings show that the HTLp assay for IL-2-secreting cells from normal subjects is sufficiently sensitive to detect minor system incompatibilities without the need for in-vivo priming. This should facilitate the molecular analysis of minor system antigens in man.

Although most patients with a high donor anti-recipient HTLp frequency developed moderate-to-severe acute GVHD, there were some exceptions. One explanation may relate to the possibility that some mH antigens are expressed in a tissue-specific manner, to the extent that the antigens expressed by peripheral blood mononuclear cells differ from those expressed by the target tissues for GVHD reactions.[20] A second possible explanation for some "inappropriately" high HTLp frequencies is a response to polymorphic proteins derived from the fetal calf or human AB sera used for the cryopreservation of cells or in the assay culture medium. It is possible that some foreign serum proteins may be processed and on occasion presented in vitro as antigenic peptides.[21]

The HTLp frequency allows the clinician to estimate, for each BMT recipient, the risk of developing moderate-to-severe GVHD, and its sequelae, after transplantation of marrow from a particular donor. Therefore, if more than one donor "matches" the patient after typing for HLA by conventional methods, the HTLp frequency could be used to select the donor with the lowest risk of initiating severe acute GVHD. If only one donor is available, the post-transplant immunosuppression could be adjusted, or the donor marrow could undergo T-lymphocyte manipulation, according to the predicted risk.

We thank Dr Walter Gregory and Dr Richard Szydlo for statistical advice and Dr Martin Howell and Ms Debbie Sage for the HLA DP typing. A. P. S. and J. M. G. are members of the Leukaemia Research Fund Centre for Adult Leukaemia at the Hammersmith Hospital.

Presented at the *Am Soc Haematol* meeting, Denver, Colorado (*Blood* 1991; **78** (suppl 1): 188a (abstr 742).

## REFERENCES

1. Beatty PG, Clift RA, Mickelson EM, et al. Marrow transplantation from related donors other than HLA-identical siblings. *N Engl J Med* 1985; **313:** 765–71.
2. Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med* 1986; **314:** 729–35.
3. Perreault C, Decary F, Brochu S, Gyger M, Belanger R, Roy D. Minor histocompatibility antigens. *Blood* 1990; **76:** 1269–80.
4. DeGast GC, Mickelson EM, Beatty PG, et al. Mixed leukocyte culture reactivity and graft-versus-host disease in HLA-identical marrow transplantation for leukemia. *Bone Marrow Transplant* 1992; **9:** 87–90.
5. Kaminski E, Hows J, Man S, et al. Prediction of graft versus host disease by frequency analysis of cytotoxic T cells after unrelated donor bone marrow transplantation. *Transplantation* 1989; **48:** 608–13.
6. Cohen J. Cytokines as mediators of graft-versus-host disease. *Bone Marrow Transplant* 1988; **3:** 193–97.
7. Civin CI. Human monomyeloid cell membrane antigens. *Exp Hematol* 1990; **18:** 461—67.
8. Bidwell JL, Bidwell EA, Savage DA, Middleton D, Klouda PT, Bradley BA. A DNA-RFLP typing system that positively identifies serologically well-defined and ill-defined HLA-DR and DQ alleles, including DRw10. *Transplantation* 1988; **45:** 640–46.
9. Howell WM, Sage DA, Haegert DG, Evans PR, Smith JL. PCR-SSO typing for HLA-DPB alleles. *Eur J Immunogenet* 1991; **18:** 81–95.
10. Clouse KA, Bach FH, Orosz CG. Detection of HSV-1-induced lymphokine production in human cells by a direct indicator cell addition assay. *J Immunol* 1984; **132:** 2961–67.
11. Gillis S, Smith KA. Long term culture of tumour-specific cytotoxic T cells. *Nature* 1977; **268:** 154–66.
12. Bishop DK, Orosz CG. Limiting dilution analysis for alloreactive, TCGF-secretory T cells: two related LDA methods that discriminate between unstimulated precursor T cells and in vivo alloactivated T cells. *Transplantation* 1989; **47:** 671–77.
13. Fazekas de St Groth S. The evaluation of limiting dilution assays. *J Immunol Methods* 1982; **49:** R11–R23.
14. Goldman JM, Apperley JF, Jones L, et al. Bone marrow transplantation for patients with chronic myeloid leukemia. *N Engl J Med* 1986; **314:** 202–07.
15. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation* 1975; **18:** 295–304.
16. Goldman JM, Gale RP, Horowitz M, et al. Bone marrow transplantation for chronic myelogenous leukemia in chronic phase: increased risk of relapse associated with T-cell depletion. *Ann Intern Med* 1988; **108:** 806–11.
17. Storb R, Buckner CD. Human bone marrow transplantation. *Eur J Clin Invest* 1990; **20:** 119–32.
18. Irle C, Beatty PG, Mickelson E, Thomas ED, Hansen JA. Alloreactive T cell responses between HLA-identical siblings: detection of anti-minor histocompatibility T cell clones induced in vivo. *Transplantation* 1985; **40:** 329–33.
19. Batchelor JR, Brookes PA, Davey NJ, et al. In vitro methods and selection of HLA-matched unrelated donors for bone marrow transplantation. *Transplant Proc* 1991; **23**: 1711–12.
20. Goulmy E, Voogt P, van Els C, de Bueger M, van Rood J. The role of minor histocompatibility antigens in GVHD and rejection: a mini-review. *Bone Marrow Transplant* 1991; 7 (suppl 1): 49—51.
21. Panina-Bordignon P, Corradin G, Roosnek E, Sette A, Lanzavecchia A. Recognition by class II alloreactive T cells of processed determinants from human serum proteins. *Science* 1991; **252:** 1548–50.

# SHORT REPORTS

## Creutzfeldt-Jakob disease and blood transfusion

T. F. G. ESMONDE R. G. WILL
J. M. SLATTERY R. KNIGHT
R. HARRIES-JONES R. DE SILVA
W. B. MATTHEWS

Epidemiological surveillance of Creutzfeldt-Jakob disease (CJD) in the UK identified 21 patients who had received a blood transfusion and 29 who had donated blood, out of a total of 202 definite and probable cases. This frequency of blood transfusion or donation did not differ from that in age and sex matched controls, and the clinical features in patients with a history of blood transfusion were similar to those of classical CJD and clearly distinct from CJD in recipients of human growth hormone. This evidence does not suggest that blood transfusion is a major risk factor for CJD.

*Lancet* 1993; **341**: 205–07.

Iatrogenic transmission has been responsible for a few cases of Creutzfeldt-Jakob disease by direct implantation in or adjacent to the central nervous system during neurosurgery, by dura mater grafts or corneal transplantation, and by peripheral inoculation during therapy with human-pituitary-derived hormones.[1] It has

been suggested that CJD might be transmitted by blood products derived from patients with CJD during the prodromal stage,[2] although CJD linked aetiologically to blood transfusion has never been recorded. Transmission of CJD to rodents by intracerebral inoculation of buffy coat and whole blood has been reported,[3,4] but the whole blood result was not replicated[5] and transfusion of 300 ml whole blood from a CJD patient to a chimpanzee has not effected transmission after 8 years.[6] Assessment of blood transfusion as a risk factor for CJD is complicated by the potentially long incubation period in iatrogenic cases, but helpful information includes the frequency of blood transfusion in CJD cases when compared with that in controls and the clinical features of CJD patients with a history of blood transfusion. Peripheral inoculation of a transmissible agent is associated with an atypical clinical presentation in pituitary-hormone-associated cases of CJD[1] and in kuru.[6] If CJD were transmissible by blood transfusion, clinical presentation would probably therefore be distinct from that of classical CJD.

Definite and probable cases of CJD were identified in the 1980–84 prospective survey of CJD in England and Wales, and in the current prospective UK national surveillance project by direct notification or from death certificates.[7] Definite and probable cases were identified by the criteria of Masters et al.[8] Information on blood transfusions was obtained from next of kin and/or case notes. Clinical features and results of investigations were also recorded. In the 1980–84 study information on a history of blood transfusion was sought in two age/sex matched hospital-based control groups, one with non-neurological conditions and the other with neurological conditions clearly distinguishable from CJD. Suitable controls were found in 92 of 123 cases.[7] In the prospective study (1990–92) information on blood transfusion was obtained from one age/sex matched hospital control group and comparative information with cases is available in 63 controls. The controls were considered appropriate for the comparison of transfusion history because all past medical and surgical events related to the current hospital admission were excluded from analysis. Clinical information on cases of CJD acquired after therapy with human pituitary extract (HgH/HGnH cases) was obtained from published case-reports and through the surveillance programme. A summary of information in 20 hormone recipients has been published[1] and is used here for comparative purposes.

16 cases of CJD with a definite history of blood transfusion were identified out of 202 definite and probable cases of CJD. A further 5 cases had a possible history of transfusion; 1 other case with a blood transfusion at the time of onset of first symptoms has been excluded from the analysis. A comparison of blood transfusion history (definite and possible) in cases and controls revealed no significant difference:

| *Group* | *Received blood* | *Donated blood* |
|---|---|---|
| *1980–84 England and Wales (n=92)* | | |
| CJD | 15 *(16%)* | 14 *(15%)* |
| Controls (combined) | 37 *(20%)* | 20 *(11%)* |
| *1990–92 UK (n=63)* | | |
| CJD | 6 *(10%)* | 10 *(16%)* |
| Controls | 9 *(14%)* | 13 *(21%)* |
| *Both studies combined* | | |
| CJD (n=155) | 21 *(14%)* | 24 *(15%)* |
| Controls (n=247) | 46 *(19%)* | 33 *(13%)* |

For all six 2×2 tables $p > 0.1$ ($\chi^2$ test).

The mean interval from blood transfusion to the onset of clinical symptoms of CJD was 174 months (median 114, SD 18, range 2–588).

24 out of 155 cases in the case-control studies had a history of blood donation and this was not significantly different from the frequency in the control groups. If we add in cases for which no control data were available, 29 patients with CJD had donated blood. In most instances blood had been donated more than 20 years before CJD developed and the number of donations was usually limited. However, 6 individuals had donated more than 10 units, including 3 individuals who had given 25–40 units and 1 who had donated about 50. We have information on geographical distribution for cases of CJD in 1970–84[9] and for those in the UK study from 1985 and there is no evidence of an increased incidence of CJD in localities where these multiple donors resided at the time of blood donation.

We have compared the clinical features of cases of CJD with a history of definite blood transfusion with those in HgH/HGnH recipients[1] and in 123 sporadic cases identified in England and Wales between 1980 and 1984. In the blood transfusion recipients and in sporadic cases, mental deterioration (often frank cognitive impairment) was the initial symptom in about two-thirds, the other one-third having a cerebellar presentation:

| | *Hormone therapy (n=20)* | | *Blood transfusion (n=16)* | | *Sporadic (n=123)* | |
|---|---|---|---|---|---|---|
| *Feature* | *Onset* | *Course* | *Onset* | *Course* | *Onset** | *Course* |
| Mental deterioration | 0% | 85% | 69% | 100% | 61% | 100% |
| Cerebellar signs | 100% | 100% | 33% | 44% | 27% | 59% |
| Visual/ oculomotor | 15% | 45% | 0% | 33% | 8% | 43% |
| Myoclonus | 0% | 80% | 0% | 88% | 2% | 83% |
| EEG periodicity | 0% | 5% | 0% | 69% | 0% | 68% |

*Not included are 13% of patients with sporadic CJD presenting with dysphasia or pyramidal/extrapyramidal signs.

In the HgH/HGnH recipients CJD always presented as a cerebellar syndrome, mental deterioration developing later if at all. Myoclonus occurred with equal frequency in all three groups but electroencephalographic periodicity was present in over two-thirds of blood transfusion and sporadic cases and in only 1 of the HgH/HGnH recipients. The clinical features in the recipients of blood transfusion were therefore consistent with sporadic CJD and were distinct from those in hormone recipients.

These data do not suggest that blood transfusion is a major risk factor for CJD. Only 16 of 202 cases of CJD had a history of definite blood transfusion and this frequency did not differ from that in matched controls. Information on blood transfusion had to be obtained from relatives and some transfusions may not have been recalled. The use of age/sex matched controls largely excludes this potential source of bias and any bias introduced by more detailed questioning of the relatives of affected cases would tend to increase, not decrease, any apparent relationship between blood transfusion and CJD. Epidemiological evidence cannot, however, exclude the possibility that isolated cases of CJD are caused by transmission of a causative agent by blood transfusion.

The predominant clinical features in CJD associated with HgH or HGnH are distinct from those in classical CJD.[1] Although there is preliminary evidence of a genetic influence on susceptibility in a few HgH recipients, this cannot explain the atypical presentation; the clinical features in all HgH/HGnH cases and in all kuru cases are atypical, regardless of genotype. The likeliest explanation for the atypical features is the route of inoculation of the agent. The clinical features in transfusion recipients with CJD are indistinguishable from those in sporadic cases with no history of blood transfusion, and that observation supports the view that blood transfusion is not a risk factor. Nevertheless, every precaution should be taken to ensure

that blood (or blood products) is not obtained from individuals with CJD or at high risk of CJD (eg, members of CJD families or people who have been treated with pituitary-extracted HgH).

The National Creutzfeldt-Jakob Disease Surveillance Programme is funded by the Department of Health and the Scottish Home and Health Department, and is only possible through the cooperation of neurologists, neuropathologists, neurophysiologists, and others throughout the UK.

## REFERENCES

1. Brown P, Preece MA, Will RG. Friendly fire in medicine: hormones, homografts, and Creutzfeldt-Jakob disease. *Lancet* 1992; **340:** 24–27.
2. Watkins AM. Creutzfeldt-Jakob disease and blood transfusion. *BMJ* 1991; **302:** 1537.
3. Tateishi J. Transmission of CJD from human blood and urine to mice. *Lancet* 1985; ii: 1074.
4. Manuelidis EE, Kim JH, Mericangas JR, Manuelidis L. Transmission to animals of Creutzfeldt-Jakob disease from human blood. *Lancet* 1985; ii: 896–97.
5. Doi T. Relationship between periodical organ distribution of Creutzfeldt-Jakob disease, CJD agent infectivity and prion protein gene expression in CJD agent-infected mice. *Nagasaki Igakkai Zasshi* 1991; **66:** 104–14.
6. Gajdusek DC. Subacute spongiform encephalopathies: transmissible cerebral amyloidoses caused by unconventional viruses. In: Fields BN, Knipe DM, eds. Fields virology, 2nd ed. New York: Raven, 1990: 2289–324.
7. Harries-Jones R, Knight R, Will RG, Cousens S, Smith PG, Matthews WB. Creutzfeldt-Jakob disease in England and Wales, 1980–1984: a case-control study of potential risk factors. *J Neurol Neurosurg Psychiatry* 1988; **51:** 1113–19.
8. Masters CL, Harris JO, Gajdusek DC, Gibbs CJ Jr, Bernoulli C, Asher DM. Creutzfeldt-Jakob disease: patterns of worldwide occurence and the significance of familial and sporadic clustering. *Ann Neurol* 1979; **5:** 177–88.
9. Cousens SN, Harries-Jones R, Knight R, Will RG, Smith PG, Matthews WB. Geographical distribution of cases of Creutzfeldt-Jakob disease in England and Wales 1970–84. *J Neurol Neurosurg Psychiatry* 1990; **53:** 459–65.

ADDRESSES: **Department of Clinical Neurosciences, Western General Hospital, Edinburgh EH4 2XU, UK** (T. F. G. Esmonde, MRCP, R. G. Will, FRCPE, J. M. Slattery, MSc, R. de Silva, MRCP); **University Department of Clinical Neurology, Radcliffe Infirmary, Oxford** (R. Knight, MRCP, Prof W. B. Matthews, FRCP); **and Department of Medicine for the Elderly, Poole Hospital** (R. Harries-Jones, MD). Correspondence to Dr R. G. Will.

# Deficient repair of DNA lesion O⁶-methylguanine in cirrhosis

JANE D. COLLIER KUNDE GUO
ALASTAIR D. BURT
MARGARET F. BASSENDINE GLENN N. MAJOR

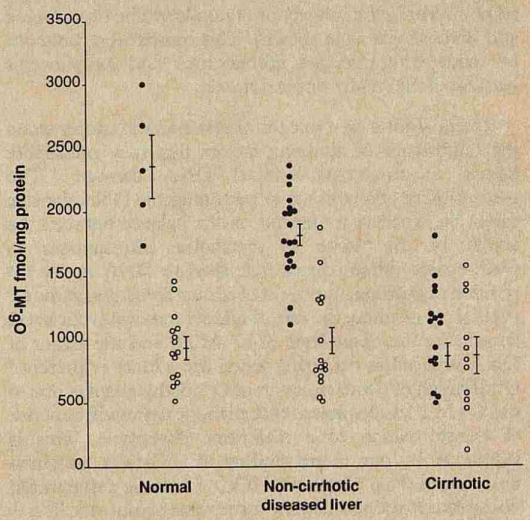
Cirrhosis is a risk factor for hepatocellular carcinoma. O⁶-methylguanine is a promutagenic and potentially carcinogenic DNA lesion produced by environmental alkylating agents. If it is not repaired, DNA replication can lead to a G-to-A transition mutation, which is a known mechanism of oncogene activation. We have found that the activity of the repairing methyltransferase enzyme is significantly lower in cirrhotic tissue than in non-cirrhotic diseased liver or in normal liver. This finding suggests a mechanism for cirrhosis being a risk factor for cancer of the liver: increased cellular proliferation together with persistence of O⁶-methylguanine might lead to malignant transformation of liver cells through mutation and oncogene activation.

*Lancet* 1993; **341**: 207–08.

Cirrhosis is a universal risk factor for hepatocellular carcinoma (HCC). Although HCC is uncommon in northern Europe it is one of the most frequent cancers worldwide. The precise nature of the relation between cirrhosis and HCC has yet to be established.[1] In view of the usual requirement for partial hepatectomy in rodent models of chemical hepatocarcinogenesis one mechanism may relate to the hepatocyte regeneration and increased cell proliferation, seen in cirrhosis, acting to promote carcinogenesis through DNA damage by environmental mutagens. N-nitroso compounds such as the alkylnitrosoureas and environmentally ubiquitous nitrosamines are potent alkylating carcinogens to which all species thus far examined are susceptible. Moreover, the liver is a very frequent site for tumours induced by these agents in animal models. O⁶-methylguanine is a powerfully promutagenic DNA base lesion produced by such agents, which, if not repaired before DNA replication, can lead to G-to-A transition mutation, a cause of malignant activation of the cellular H-*ras* oncogene and disturbance of p53 tumour suppressor function in animal models.[2,3]

O⁶-methylguanine is repaired by O⁶-methylguanine-DNA methytransferase (O⁶-MT), which transfers the methyl group from the O⁶ position of guanine to one of its cysteine residues, thereby restoring native guanine in DNA.[2] As a result of this automethylation, O⁶-MT becomes inactivated, and, unusually for an enzyme, is not regenerated. Thus, in the absence of new enzyme synthesis, the total level of O⁶-MT represents a cell's DNA repair capacity for this promutagenic lesion. Might, therefore, O⁶-methylguanine repair by O⁶-MT be deficient in cirrhotic liver?

Liver biopsy tissue was studied from 40 patients admitted for investigation of liver disease and subsequently classified as having cirrhosis (alcoholic = 9, cryptogenic = 4, autoimmune = 2, hepatitis C = 1) or non-cirrhotic liver disease (alcoholic steatosis = 13, idiopathic steatosis = 6) or normal liver histology (5). Lymphocytes were isolated from venous blood, at the time of liver biopsy, in 30 patients, and from 11 healthy controls with no biopsies. Extracts of




**Liver and lymphocyte O⁶-MT levels related to total extract protein.**

Individual data with mean (SE); ● = liver, ○ = lymphocytes.

5-15 mg samples of liver, stored at −80°C, were prepared in essentially the same manner as previously reported for human spleen,[4] except for the use of microhomogenisation and omission of re-extraction of homogenate particles and sonication steps. The extract routinely contained over 90% of the O⁶-MT present in the biopsy homogenate. Lymphocyte extracts were prepared in the same way, except that homogenisation was done in buffer made 50 mmol/l with NaCl to achieve extraction efficiency equivalent to that obtained with liver tissue. O⁶-MT levels were determined by a sensitive direct enzyme assay[4] and were expressed in relation to extract DNA and protein content; since the protein/DNA ratio differs between lymphocyte and liver extracts (data not shown) DNA determination in liver should more accurately reflect cellularity of this tissue, facilitating direct comparison with lymphocyte enzyme levels. Protein and DNA were assayed by the methods of Bradford[5] and Cesarone et al.[6]

Analysis of variance (*F* test) was used to compare differences in tissue O⁶-MT levels between groups of patients whose mean enzyme levels were compared by *t*-test. Lymphocyte and liver levels of O⁶-MT from the same patient were compared by linear regression analysis.

We found highly significant differences in liver O⁶-MT between the three groups, the mean activity being lower in the cirrhotic group than in patients with non-cirrhotic liver disease and in healthy controls. This was true whether activities were expressed in relation to protein or DNA, but lymphocyte levels did not differ significantly:

| $O^6$-MT | Normal | Non-cirrhotic | Cirrhotic |
|---|---|---|---|
| *Liver** | (n=5) | (n=19) | (n=16) |
| fmol/μg DNA | 885 (49) | 870 (68) | 548 (61) |
| fmol/mg protein | 2364 (222) | 1823 (75) | 1056 (92) |
| *Lymphocytes* | (n=15) | (n=15) | (n=11) |
| fmol/μg DNA | 1138 (92) | 1449 (128) | 1193 (74) |
| fmol/mg protein | 943 (71) | 985 (105) | 872 (137) |

*Significant differences within three groups ($F=32$, $p=0.0001$ for "protein"; $F=7.53$, $p=0.0018$ for "DNA") and between cirrhosis and both non-cirrhosis ($p=0.0001$; $p=0.0014$) and normal ($p=0.0001$; $p=0.008$).

There was a two-fold inter-individual variation in combined normal and non-cirrhotic liver levels, and a four-fold inter-individual variation in cirrhotic liver levels (figure). We found no correlation between O⁶-MT levels in lymphocytes and liver from individuals in all three groups combined or in the subgroups of non-cirrhotic liver disease and cirrhosis (data not shown). This comparison could not be made with matched normal liver and lymphocytes because of the small sample number.

Recent studies on a role for nitrosamines in cancer stress the importance of studying factors that may predispose tissues to carcinogen-induced DNA damage.[7] The susceptibility of any tissue to promutagenic DNA damage might be expected to depend on the balance between the ability of the tissue to metabolise nitrosamines to carcinogenic intermediates that alkylate DNA at the O⁶ position of guanine, the extent of adduct formation at the O⁶ position of guanine, the rate of adduct removal (which will depend on the tissue level of O⁶-MT), and the extent of DNA replication occurring when the adduct is present.[8] Our finding of deficient repair of O⁶-methylguanine due to low O⁶-MT levels suggests that cirrhotic tissue will be at risk of transformation to a malignant phenotype. This is supported by our recent finding of a G-to-A transition mutation in the p 53 gene in HCC.[9] Cirrhotic tissue would be expected to be additionally susceptible because the liver is an important site of nitrosamine metabolism, and cirrhosis is characterised by increased liver-cell proliferation and DNA synthesis. The reported correlation of O⁶-MT levels in gastric mucosa and in circulating lymphocytes suggested that lymphocyte enzyme levels might be a surrogate for protein in less accessible tissues.[10] We found no correlation between diseased liver and lymphocytes, suggesting that the differences seen in O⁶-MT levels in liver are disease and target-tissue-specific.

Our observation of greater inter-individual variation in diseased liver than normal tissue raises the possibility that it is a combination of genetic and acquired factors that determines O⁶-MT levels in disease. Variable O⁶-MT levels in cirrhotic tissue imply that not all patients will carry the same risk of HCC. This is consistent with cirrhosis of different aetiology displaying a characteristic risk for development of HCC[1].

Our finding, implying deficient DNA repair, suggests a mechanism to explain the role of cirrhosis in the multistage process of hepatocarcinogenesis: increased cell proliferation, together with persistence of O⁶-methylguanine, might lead to a specific mutation causing malignant oncogene activation and/or tumour suppressor gene inactivation.

We thank Dawn Ross for assistance with lymphocyte preparations, Dr Peter Kelly (department of medical statistics, University of Newcastle upon Tyne) for guidance on statistical methods, and Prof Oliver F. W. James for providing some of the patients. J. D. C. is supported by a grant from the North of England Cancer Research Campaign and K. G. by funds from the Newcastle upon Tyne District Health Authority Research Committee.

## REFERENCES

1. Bassendine MF. Aetiological factors in hepatocellular cancer. *Baillière's Clin Gastroenterol* 1987; **1:** 1–16.
2. Pegg AE. Mammalian O⁶-alkylguanine-DNA alkyltransferase: Regulation and importance in response to alkylating carcinogenic and therapeutic agents. *Cancer Res* 1990; **50:** 6119–29.
3. Ohgaki H, Hard GC, Hirota N, Maekawa A, Takahashi M, Kleihues P. Selective mutation of codons 204 and 213 of the p 53 gene in rat tumors induced by alkylating N-nitroso compounds. *Cancer Res* 1992; **52:** 2995–98.
4. Major GN, Gardner EJ, Lawley PD. Direct assay for O⁶-methylguanine-DNA methyltransferase and comparison of detection methods for the methylated enzyme in polyacrylamide gels and electroblots. *Biochem J* 1991; **277:** 89–96.
5. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. *Anal Biochem* 1976; **72:** 248–245.
6. Cesarone C, Bolgnesi C, Santi L. Improved microfluorometric DNA determination in biological material using 33258 Hoechst. *Anal Biochem* 1979; **102:** 188–97.
7. Cheng KK, Day NE, Duffy SW, Lam TH, Fok M, Wong J. Pickled vegetables in the aetiology of oesophageal cancer in Hong Kong Chinese. *Lancet* 1992; **339:** 1314–18.
8. Craddock VM. The relative importance of DNA replication, DNA repair and metabolic activation in induction of cancer by *N*-nitroso compounds. Oslo: Norwegian University Press, 1986: 242–56.
9. Challen C, Lunec J, Warren W, Collier J, Bassendine MF. Analysis of the p53 tumour suppressor gene in hepatocellular carcinomas from Britain. *Hepatology* (in press).
10. Kyrtopoulos SA, Ampatzi P, Davaris P, Haritopoulos N, Golematis B. Studies in gastric carcinogenesis IV: O⁶-methylguanine and its repair in normal and atrophic biopsy specimens of human gastric mucosa. Correlation of O⁶-alkylguanine-DNA alkyltransferase activities in gastric mucosa and circulating lymphocytes. *Carcinogenesis* 1990; **11:** 431–36.

ADDRESSES: **Departments of Medicine** (J. D. Collier, MRCP, K. Guo, PhD, M. F. Bassendine, FRCP, G. N. Major, PhD), **and Pathology** (A. D. Burt, MD), **Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, UK.** Correspondence to Dr Glenn N. Major, Medical Molecular Biology Group, Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4HH, UK.