Cell adhesion molecule expression in the
dorsolateral prefrontal cortex and anterior
cingulate cortex in major depression in the elderly

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and JOHN T. O'BRIEN

Background Neuroimaging studies have demonstrated changes in the
dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) in
major depression.

Aims We investigated the expression of cell adhesion molecules (CAMs) in the
prefrontal cortex in depression.

Method Immunohistochemistry to localise CAMs in post-mortem tissue from
20 subjects with major depression and 20 controls, and image analysis to quantify
their expression.

Results We found significant increases in CAMs in the grey matter of the DLPFC
in the depression group but no comparable differences in the ACC or occipital cortex. In the white matter there
was a non-significant increase in intercellular adhesion molecule-1 in the
DLPFC in the depression group but no increase in the other areas or for vascular
cell adhesion molecule-1 in any area. Paired
tests showed specificity for the DLPFC in the depression group only.

Conclusions The increase in CAM expression in the DLPFC suggests an
inflammatory reaction and is consistent with ischaemia.

Declaration of interest None.

The ‘vascular depression’ hypothesis proposes that late-life depression is associated
with vascular disease affecting the frontal-subcortical circuitry, based on associations
between depression and vascular disease (Alexopoulos et al., 1997) and magnetic
resonance imaging studies in major depression, which have shown an increase in white
matter lesions (WMLs) that may have a vascular origin (O'Brien et al., 1996). The
WMLs appear to be most strongly associated with depression when they involve
frontal-subcortical circuits, which reciprocally link prefrontal areas (the dorsolateral
prefrontal cortex (DLPFC) and the anterior cingulate cortex (ACC)) to the basal ganglia
(Greenwald et al., 1998). This is consistent with positron emission tomography studies in
depression, which have demonstrated hypometabolism in the DLPFC (Bench et al., 1992) and the ACC (Drevets et al.,
1997). This study tested whether ischaemic changes occur in the brain in depression in
the DLPFC and the ACC, by measuring two cell adhesion molecules (CAMs). The
expression of these CAMs is increased by ischaemia in vitro (Kim, 1996) and in
human cerebral microvessels in the vicinity of the infarct following ischaemic stroke
(Lindsberg et al., 1996).

METHOD

Subjects
Brain tissue from 40 subjects was obtained from the Neuropathology Department/
Newcastle Brain Tissue Bank. Permission had been given for post-mortem research,
etical approval was granted for this study. Cases consisted of 20 subjects who
had had depression and 20 controls. Subjects with depression were included if they
were 60 years or over at death and had suffered at least one well-documented
episode of DSM–IV major depression (American Psychiatric Association, 1994).
Subjects were excluded if they had ever
met the DSM–IV criteria for dementia, schizophrenia or other psychotic disorders,
a manic or hypomanic episode or a mood disorder due to a general medical
condition. Subjects were also excluded if they met the neuropathological criteria for any
known cause of dementia (such as Alzheimer’s disease, vascular dementia or demen-
tia with Lewy bodies) or had evidence of any other neurological disorder. Control
subjects were known to be capable of living independently and met the same criteria
except that they had never suffered a depressive episode.

All subjects in the depression group had undergone extensive assessments, including
a full history, mental state examination, physical examination, screening blood tests,
cognitive tests and, in some cases, computed tomodraphy or magnetic resonance imaging
scans. All had received standard antidepressant treatment regimes, with selective
serotonin reuptake inhibitors or tricyclic antidepressants singly or often in combina-
tion with other agents, and 11 had received electroconvulsive therapy. No control sub-
ject had taken any antidepressant or anti-
psychotic medication. The case notes on
all subjects were examined to see if they
had a history of hypertension sufficient to
need treatment with antihypertensive
medication. All subjects had had a full
post-mortem assessment (except one whose
body was unavailable for autopsy) and the
post-mortem delay was recorded.

Tissue
After death the right hemisphere was fixed
in 10% formalin and the brains were dis-
sected in a standard manner. To obtain tis-
sue blocks for analysis, tissue was selected
from three areas: the DLPFC (Brodman
areas (BA) 9 and 46), the ACC (BA 24)
and the occipital cortex (BA 19 and 39) as
a comparison area. The DLPFC blocks
were chosen by carefully selecting the coro-
nal slice from each subject to include BA 9
and 46 according to a standard map (Perry,
1993). Owing to the variation in humans,
this may have included BA 10 in some sub-
jects. The ACC block was taken from BA
24 just rostral to the genu of the corpus
callosum or, occasionally, just including
the rostral tip of the genu. These blocks
were chosen to examine the areas identified
as reduced in function in depression in the
DLPFC (Bench et al., 1992) and ACC
(Drevets et al., 1997). The occipital cortex
block was chosen to determine whether
any changes in the prefrontal areas were specific for these areas or occurred throughout the brain. These blocks were embedded in paraffin wax, the duration of fixation of these large blocks was recorded and they were sectioned using a sledge microtome into 10-μm sections (one per subject) on large slides (3 × 2”). These slides were coded so that all analysis could be carried out blind to diagnosis.

### Immunocytochemistry
Sections were processed for immunocytochemical localisation in a standard manner. Briefly, sections were dewaxed in xylene, rehydrated and microwaved in 0.01% citrate buffer (pH 6.0) to optimise antigen retrieval. They were immersed in hydrogen peroxide, blocked with an appropriate serum and incubated for 1 h at room temperature with the primary antibody. The primary antibodies used were a polyclonal antibody to intercellular adhesion molecule-1 (ICAM-1) (R&D Systems; 1:500 dilution), a polyclonal antibody to vascular cell adhesion molecule-1 (VCAM-1) (R&D Systems; 1:800 dilution) and a monoclonal antibody to collagen IV (Sigma; 1:500 dilution). Immunocytochemistry was carried out using slides with code numbers to ensure blindnedness and with a random order of depression and control cases. Slides were processed together for each primary antibody in order to ensure that the immunocytochemistry conditions were the same for both groups. The expression of ICAM-1 and VCAM-1 is increased by ischaemia (Kim, 1996) in cerebral endothelial cells in vitro and studies of human ischaemic stroke have also shown increased ICAM-1 expression in the microvessels in association with the stroke lesions (Lindsberg et al., 1996). Both ICAM-1 and VCAM-1 were therefore chosen as putative markers of cerebral ischaemia. Collagen IV (a basement membrane protein) is a marker of the density of the microvascular tree and was measured to determine whether any differences in ICAM-1 or VCAM-1 reflected alterations in the whole vascular tree rather than in ICAM-1 or VCAM-1 expression in the vessels (Kalaria & Hedera, 1995). Appropriate secondary antibodies were then applied followed by avidin-biotinylated horseradish peroxidase complex (Vector Laboratories Ltd) and diaminobenzidine (DAB) as a chromagen. All sections were lightly counterstained with haematoxylin and were examined using a light microscope to check the quality of staining before proceeding to quantitative analysis.

### Quantitative analysis
Analysis was conducted on one section per anatomical area per subject, based on calculations of the variability between fields and between different immunocytochemical assays. The mean coefficient of error for five fields (from five subjects) was 6.8% and there was very little difference whether fields were taken from one section per subject (mean coefficient of error = 6.6% for ten fields from five subjects) or from two sections per subject run in different immunocytochemistry assays (mean coefficient of error = 7.3% for ten fields, five per section, from five subjects).

Images were captured using a 10 objective lens on a Zeiss Axiosplan 2 light microscope coupled to a three-chip CCD true-colour video camera (JVC KY-F55B), producing a field size of 185 000 μm² (0.185 mm²). For each antibody, five images selected randomly were captured from the grey matter and ten from the white matter on each section from the DLPCF and the occipital cortex, and ten from the grey matter and fifteen from the white matter in the ACC. Images were obtained randomly by the operator selecting a field by moving the stage while not looking at the section. If the field was outside the area of interest (grey or white matter) then this was repeated until the field included the required tissue. This ensured that each field was selected independently of the microscopic appearance of its microvessels. They were analysed blind to diagnosis on a monitor using a standard software program (Image Pro Plus, version 4.0; Media Cybernetics). This involved measuring the area of DAB staining, expressed as a percentage of the total image area (areal fraction), and then calculating a mean score for the grey and white matter for each cortical area. The areal fraction was not obtained using thresholding because this method was found to lead to inappropriate inclusion of structures that were not stained with the CAM antibodies. Instead, the dropper method was used, in which the operator selected DAB-stained microvessels by eye in each field according to their exact red–green–blue colour characteristics; this avoided the thresholding problem of including other structures. Interrater and intrarater reliability ratings were calculated (from 48 images on 16 patients) for the two raters using the intraclass correlation coefficient and the coefficient of variation, respectively.

### Statistical analysis
Statistical comparisons were carried out using SPSS software (Version 9.0). Tests for normality were conducted and unpaired, two-way Student’s t-tests or Mann–Whitney tests were used, as appropriate, to compare ICAM-1 and VCAM-1 expression in the depression and control groups. To examine whether any changes in ICAM-1 and VCAM-1 expression showed specificity for the prefrontal areas, paired t-tests and Wilcoxon signed rank tests were used, as appropriate, for within-group comparisons. Secondary analyses were conducted to examine possible confounders. These included comparison of collagen IV expression in the two groups and possible effects of treatment and hypertension on CAM expression. Pearson correlation coefficients, t-tests or Mann–Whitney tests, and analysis of variance were used, as appropriate, for these analyses.

### RESULTS
Subjects’ characteristics are given in Table 1, which shows no significant differences in age, post-mortem delay or duration of fixation between the two groups. The coefficients of variation were 3.9% for rater 1 and 9.8% for rater 2, and the intra-class correlation coefficient was 0.97. Figures 1 and 2 show ICAM-1 and VCAM-1 expression in the two groups and Figures 3 and 4 show ICAM-1 and VCAM-1 immunoreactivity, respectively.

### Comparison of depression and control groups
The ICAM-1 expression was significantly higher in the depression group in both the grey matter (t=2.77, d.f.=38, P=0.009) and the white matter (t=2.28, d.f.=38, P=0.029) in the DLPCF, but not in the ACC (grey matter: t=0.86, d.f.=38, P=0.394; white matter: t=1.15, d.f.=38, P=0.258). The VCAM-1 expression also was significantly higher in the grey matter of the DLPCF (W=321, P=0.017) but not in the white matter (W=362, P=0.199). In the occipital cortex, ICAM-1 was significantly increased in depression in the
grey and white matter, because we had ICAM-1 and VCAM-1 or for assessing ICAM-1 and VCAM-1. Such significant for both ICAM-1 and VCAM-1. Such grey matter of the DLPFC remained significant for both ICAM-1 and VCAM-1. Such areas in each subject, and the increase in the grey matter was set at 0.017, based on testing three W W d.f.

Table 1 Characteristics of the depression and control groups

<table>
<thead>
<tr>
<th></th>
<th>Depression group (n=20)</th>
<th>Control group (n=20)</th>
<th>Test statistic</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean (s.d.))</td>
<td>75.0 (7.37)</td>
<td>74.2 (7.46)</td>
<td>0.30</td>
<td>0.77</td>
<td>−5.45 to 4.05</td>
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<tr>
<td>Gender (M/F)</td>
<td>7/13</td>
<td>7/13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset of depression, years (mean (s.d.))</td>
<td>63.8 (14.83)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of fixation, months (mean (s.d.))</td>
<td>123.9 (59.68)</td>
<td>115.0 (59.26)</td>
<td>0.47</td>
<td>0.64</td>
<td>−46.97 to 29.17</td>
</tr>
<tr>
<td>Post-mortem delay, hours (mean (s.d.))</td>
<td>34.6 (22.72)</td>
<td>28.0 (16.18)</td>
<td>1.05</td>
<td>0.30</td>
<td>−19.18 to 6.08</td>
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<tr>
<td>Causes of death</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac</td>
<td>7</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>7</td>
<td>3</td>
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<tr>
<td>Carcinoma</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Other cause</td>
<td>1^</td>
<td>3^</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Renal failure.
2. Haematemesis, mesenteric infarction and liver failure.

Fig. 1 Inter cellular adhesion molecule-1 (ICAM-1) expression in three cortical areas in major depression.

In the grey matter, paired t-tests showed that ICAM-1 expression in the DLPFC in the depression subjects was highly significantly elevated compared with both the ACC (t=3.05, P=0.01) and the occipital cortex (t=2.72, P=0.01), whereas there was no difference between the ACC and the occipital cortex (t=1.77, P=0.09). In contrast, in grey matter in the control group there were no significant differences between the DLPFC and the ACC (t=1.26, P=0.22), the DLPFC and the occipital cortex (t=1.71, P=0.10) or the ACC and the occipital cortex (t=0.02, P=0.98). In the white matter of the control group there was a significant increase in ICAM-1 in the DLPFC compared with the ACC (t=2.59, P=0.02) but no significant differences between the DLPFC and the occipital cortex (t=1.76, P=0.10) or between the ACC and the occipital cortex (t=1.39, P=0.18). Similarly for VCAM-1, there was a significant increase in the grey matter of the depression group in the DLPFC compared with both the ACC (Z=−3.44, P=0.001) and the occipital cortex (Z=−1.98, P=0.05) but not in the ACC compared with the occipital cortex (Z=−1.34, P=0.18). In the white matter of the depression subjects VCAM-1 was highly significantly elevated in the DLPFC compared with both the ACC (Z=−3.58, P<0.001) and the occipital cortex (Z=−3.06, P=0.002) but there was no difference in VCAM-1 expression between the ACC and occipital cortex (Z=−1.53, P=0.13). In contrast, in the control subjects there were no differences in VCAM-1 expression in the grey matter between the DLPFC and the ACC (Z=−1.32, P=0.19), the DLPFC and the
Vascular cell adhesion molecule-1 (VCAM-1) expression in three cortical areas in major depression.

**Fig. 2**

Vascular cell adhesion molecule-1 (VCAM-1) expression in three cortical areas in major depression. Bars represent 50 μm. Arrows indicate microvessels.

**Fig. 3**

Intercellular adhesion molecule-1 expression (sections have been counterstained with haematoxylin): (a) control grey; (b) depression grey; (c) control white; and (d) depression white. Bars represent 50 μm.

Arrows indicate microvessels.

Assessment of potential confounders

There were no significant group differences in the expression of collagen IV in any area in either grey matter \( (t < 0.85, \text{d.f.} = 38, P = 0.399) \) or white matter \( (t < 1.43, \text{d.f.} = 38, P = 0.16) \). In the total group studied there was no correlation between age, post-mortem delay or duration of fixation and any of the four significant results (age: \( r < 0.055, n = 40, P > 0.56 \); post-mortem delay: \( r < 0.14, n = 40, P > 0.37 \); fixation: \( r < 0.233, n = 40, P > 0.148 \)). Hypertension did not account for the group differences (adjusted \( R^2 \) for contribution of hypertension <0.053) and a post hoc comparison of those with and without hypertension (regardless of whether or not they had depression) found no group differences for either ICAM-1 \( (t < 1.07, \text{d.f.} = 30, P > 0.291) \) or VCAM-1 \( (t < 0.78, \text{d.f.} = 30, P > 0.439) \) in any area for either grey or white matter.

In the depression group we found no correlation between age of onset of depression or any of the four significant outcome measures \( (r < 0.224, n = 20, P > 0.343) \). The group had a late age of onset of depression (see Table 1), with only six subjects having onset before 60 years and only three before 45 years. Using Mann–Whitney tests there was no difference between those who had received \( (n = 11) \) and those who had not received \( (n = 9) \) electroconvulsive therapy \( (W > 108, P > 0.552) \), nor was there any difference between those who had \( (n = 13) \) and those who had not \( (n = 7) \) received antidepressant treatment within a week of death \( (W > 60, P > 0.275) \). Two suicides were included in the depression group and none of the results was changed by re-analysis without their inclusion.

**DISCUSSION**

**Key findings**

Our main findings were of increased ICAM-1 and VCAM-1 expression in the DLPFC in the depression group. No differences in the expression of either CAM were found in the ACC and only a very modest and non-significant increase in ICAM-1 was found in the grey matter in the occipital cortex. This increased expression of ICAM-1 and VCAM-1 showed marked specificity for the DLPFC in the depression group but little specificity in the control group. These results are consistent with the hypothesis that there are ischaemia-induced inflammatory changes in the prefrontal cortex in depression.

Increase in CAM expression is consistent with ischaemia

We chose to test the vascular depression hypothesis (Alexopoulos et al, 1997) by
measuring the expression of ICAM-1 and VCAM-1 in the prefrontal cortex because these two molecules are increased by ischaemia in vitro (Kim, 1996) and studies of human ischaemic stroke have also shown increased ICAM-1 expression in the microvessels in association with the stroke lesions (Lindsberg et al., 1996). There is increasing evidence that ischaemic brain damage develops over a much longer period than was previously believed (Stoll et al., 1998) and our findings are therefore comparable with a chronic reduction in cerebral perfusion to the DLPFC as well as with acute events. A recent study in the DLPFC suggested that elderly people with depression have increased astrocytes (Miguel-Hidalgo et al., 2000), which would be consistent with our finding of increased CAM expression in the DLPFC because post-ischaemic inflammatory changes would increase astrocyte activity. Studies in younger patients with depression have reported a reduction in glia in both the ACC (Ongur et al., 1998) and the DLPFC (Rajkowska et al., 1999), which could lead to a lack of glial support for neurons and increased neuronal vulnerability. In elderly subjects chronic and/or acute ischaemia could lead to an inflammatory response causing neuronal damage and subsequent increased glial activity. However, the extent to which these findings can be generalised to late-life depression in general is unclear; our depression subjects were all hospital patients who had had multiple episodes of depression.

**Importance of DLPFC in depression in the elderly**

Our early findings (Thomas et al., 2000) showed increased ICAM-1 expression in the DLPFC in depression. We have now extended this by replicating the finding with VCAM-1 and found such increased immunoreactivity to show some specificity for the DLPFC that is not found in the ACC. One possible explanation for this pattern is that the depressive symptomatology in our subjects was produced by disproportionate dysfunction in the frontal-subcortical circuit linking the DLPFC to the head of the caudate nucleus rather than in the circuit linking the ACC to the nucleus accumbens (Alexander et al., 1986). Alternatively, because the DLPFC lies in an area of the prefrontal cortex between the

**Other possible explanations for the findings**

Although we carried out the study to test for evidence of ischaemia, we cannot exclude the possibility that depression mediates increases in CAM expression by other mechanisms, although the weight of evidence associating depression with vascular diseases, especially in the elderly (Alexopoulos et al., 1997), favours prefrontal ischaemia as the most likely explanation of the current results. Another possible pathway leading to increased CAM expression could be the increased circulating levels of cytokines (e.g. interleukin 1 and tumour necrosis factor alpha), which have also been described in depression (Connor & Leonard, 1998). These cytokines stimulate the expression of CAMs and thus form a possible link.

The two groups were similar in age, gender, post-mortem delay and duration of tissue fixation, and our analysis of the data found no evidence that the results could be explained by any of these factors. In a previous study (Thomas et al., 2001) we found no differences in clinical measures of vascular risk (e.g. hypertension) between the two groups and so the results do not appear to be due to such confounders. Age of onset of depression did not affect the results, but because most of the depression group had a late age of onset we could not fully explore the extent to which increased CAM expression might differ in an early-onset group. We also found no evidence that treatments unique to the depression group (antidepressants and electroconvulsive therapy) could account for the group differences. Because this was a case-note study we have been unable to examine fully all possible confounding factors and it is possible that the up-regulation of the CAMs was due to some other factor or factors that we have been unable to exclude as a confounder. Increased CAM expression occurs in association with amyloid plaques in Alzheimer’s disease and after stroke (Kim, 1996), but our neuropathological assessment has excluded such confounders as explanations. We measured the expression of collagen IV to examine the possibility that any differences in CAM expression were due to quantitative differences in the cerebral endothelium (Kalaria & Hedera, 1995) but because no differences in collagen IV were found we have ruled out this possible explanation as well.

![Fig. 4 Expression of vascular cell adhesion molecule-1 (sections have been counterstained with haematoxylin). (a) control grey; (b) depression grey; (c) control white; and (d) depression white. Bars represent 50 μm. Arrows indicate microvessels.](image-url)
territories of the anterior and middle cerebral arteries, it appears to be more vulnerable to ischaemia, especially owing to haemodynamic alterations (Chui & Willis, 1999). Our results are consistent with this because we found a higher CAM immunoactivity in the DLPCF in both groups, but significantly more so for subjects with depression. These two alternatives could be related because ischaemic damage to the DLPCF would produce a characteristic pattern of depressive symptomatology involving retardation, poor concentration and dysexecutive failure. Such a pattern of executive dysfunction has been associated with poor response to antidepressant treatment and with relapse and recurrence in elderly subjects with depression (Alexopoulos et al., 2000), which suggests that our findings have clinical importance.

Although further investigation is required, our findings potentially have major implications for the aetiology and management of depression in the elderly. If further research shows that post-ischaemic inflammation is involved in late-life depression, the use of anti-inflammatory treatments (e.g. non-steroidal anti-inflammatory agents or Cox-2 inhibitors) may become indicated to reduce inflammation and prevent further tissue injury.

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REFERENCES


CLINICAL IMPLICATIONS

- Inflammatory changes consistent with cerebral ischaemia were associated with depression in our elderly subjects.

- These changes showed specificity for the dorsolateral prefrontal cortex, implying that dysfunction here is particularly associated with depression in the elderly.

- This opens up the possibility for novel (anti-inflammatory and antivascular) treatments for late-life depression if these findings are confirmed.

LIMITATIONS

- Since this was a retrospective post-mortem study we were not able to examine fully the possible confounders.

- Our subjects were all elderly (over 60 years old) and the extent to which our results may apply to younger adults is not clear and needs further investigation.

- From our results we cannot determine causality; that is, whether depression leads to ischaemic changes or vice versa.

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