Volume 217, number 1, 62–64 FEB 04773 June 1987

Antibodies to normal and Alzheimer human brain structures from non-immunised mice of various ages

James Simpson, Ian H. Milne*, John O. Gardner*, Celia M. Yates, Keith James* and George Fink

MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ and **Department of Surgery, University of Edinburgh, Edinburgh, Scotland*

Received 1 April 1987

Supernatants from mouse spleen hybridoma lines established without previous immunisation were screened immunohistochemically against cryostat sections of human temporal cortex and found to stain a variety of brain structures, including Alzheimer plaques and tangles. The age of the mice had no effect on antibody production.

Immunohistochemistry; Aging; Alzheimer-type dementia; Senile plaque; Neurofibrillary tangle; (Mouse hybridoma)

1. INTRODUCTION

Virus-transformed lymphocytes from normal and ATD subjects spontaneously produce antibodies to various human brain components, including the senile plaques and neurofibrillary tangles found in large numbers in ATD [1,2]. This, together with the observation that germ-free, neonatal mice spontaneously produce antibodies to intracellular structures [3], suggests that immunisation with exogenous antigen may not be necessary for the production of antibodies. We have investigated this possibility further by studying the reactivity of antibodies from non-immunised mice of various ages to sections of normal and ATD brain.

Correspondence address: J. Simpson, MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ, Scotland

Abbreviations: ATD, Alzheimer-type dementia; BRA, brain-reactive antibody

2. MATERIALS AND METHODS

Hybridoma cell lines were prepared from mouse (Balb/c strain) spleen cells by fusion with a non-secretory myeloma line [4,5]. After 11–20 days growth starting with 2.2×10^5 cells/well, the culture supernatants were screened immunohistochemically for reactivity with acetone-fixed cryostat sections of fresh-frozen temporal cortex from Alzheimer and control brain [1] using a combination of biotinylated anti-mouse IgM and anti-mouse IgG as the secondary antibody.

3. RESULTS

Seventeen fusions yielded 722 mouse hybridoma cell lines (table 1). Eighty-seven lines (12%) produced antibodies which stained brain structures, with 24 staining more than one type of structure. Three lines examined for Ig typing were found to be IgM-producing, in line with previous results [1]. Supernatants from 42 lines stained neuronal elements, 30 stained fibrous astrocytes, 15 stained nuclei, 12 stained blood vessels, 3 stained plaques,

Published by Elsevier Science Publishers B.V. (Biomedical Division)

Table 1

Brain-reactive cell lines from mouse spleen fusions

Fusion	Mouse age (days)	No.of wells	No.of hybrids	No.of brain-reactive hybrids (%)
IM20	1	240	55	0 (0)
IM38	1	60	53	1 (2)
IM26[a]	13	360	27	2 (7)
IM27[a]	13	60	15	6 (40)
IM30[a]	24	480	96	9 (9)
IM32[a]	24	450	0	0 (0)
IM13	77	600	12	1 (8)
IM15	77	960	83	26 (31)
IM16	90	360	4	0 (0)
IM22	90	360	2	0 (0)
IM23	90	480	27	1 (4)
IM36[a]	90	360	150	21 (14)
IM37[a]	90	390	0	0 (0)
IM21	113	540	0	0 (0)
IM18	189	540	122	17 (14)
IM19	189	340	76	3 (4)
IM17	240	960	0	0 (0)

[a] Half of the spleen cells were grown as normal (IM26, 30, 36), half were preincubated with 2.5 μg/ml pokeweed mitogen (IM27, 32) or 1 mg/ml muramyldipeptide (IM37) and 0.3% 2-mercaptoethanol to stimulate antibody production. One spleen was used in each experiment, except for IM20 (9 spleens), IM26/27 (5 spleens) and IM30/32 (6 spleens)

3 stained tangles, and 1 stained an intraneuronal structure tentatively identified as lipofuscin, an age-related pigment [6]. The age of the mice had no marked effect on hybrid numbers or numbers of BRAs, although it was noteworthy that only 1 out of 108 lines from 1-day-old mice produced BRAs. The highest percentage of BRAs was obtained from a line which was stimulated in culture prior to fusion (IM27), although this was not reproducible (IM32 and IM37, table 1).

The sections with putative plaque and tangle staining were counterstained with Congo red [7] and viewed under cross-polarisation microscopy to confirm the identity of the stained elements. Two anti-plaque lines stained all plaques in sections of ATD temporal cortex in a manner similar to a human line [1], the third line appeared to stain neuronal elements in neuritic plaques. The anti-tangle lines stained tangles in perikarya and tangle material within neurites in the neuropil and plaques in sections of ATD brain.

4. DISCUSSION

We have demonstrated that immunisation of mice with exogenous antigen is not necessary for the production of antibodies which recognise human brain structures. The structures recognised by the mouse hybridoma antibodies resembled those stained by antibodies from virus-transformed human lymphocytes [1] in that neurons, astrocytes and nuclei were most commonly stained (blood vessel staining was not recorded in our previous study because of artifacts arising from the use of anti-human IgG).

The present study attempted to resolve the question of whether the age of the donor animal influences BRA production, since in our previous study [1] lymphocytes were obtained from only elderly humans. Only one hybridoma line from the 1-day-old mice produced BRAs, suggesting that gradual exposure to circulating antigens may be necessary for antibody expression, however, older mice also produced few or no BRAs (table 1), and overall there was no obvious effect of age on BRA production. This is in contrast to the finding that in mice and humans the number of serum BRAs increases with age [8]. These observations, coupled with the finding that autoantibodies are produced in neonatal mice [3], suggest that there is a basal production of antibodies in newborn animals which is increased with aging. This increase was not observed in the present study, probably because the regulation of the production of serum antibodies is different to that of antibodies from hybridoma cell lines.

Since plaques are found in mouse brain only after infection with scrapie [9], and tangles are unique to human brain, it was surprising that these structures were recognised by antibodies produced spontaneously by mouse hybridomas. The expression of these antibodies in non-immunised mice suggests that plaques and tangles contain large amounts of normal brain epitopes, or epitopes which are also found in peripheral or environmental antigens. Thus mouse hybridomas may be used to produce antibodies recognising a variety of normal and abnormal human brain structures without

the necessity of immunisation with exogenous antigen.

ACKNOWLEDGEMENTS

We thank James Treanor and Tim Sherratt for skillful technical assistance, Dr A. Gordon for neuropathological assessment of the ATD and control cases, and Christine Young for preparing the manuscript.

REFERENCES

[1] Simpson, J., Boyd, J.E., Yates, C.M., Christie, J.E., Fink, G., James, K. and Gordon, A. (1986) J. Neuroimmunol. 13, 1–8.
[2] Gaskin, F., Kingsley, B.S. and Fu, S.M. (1987) J. Exp. Med. 165, 245–250.
[3] Underwood, J.R., Pedersen, J.S., Chalmers, P.J. and Toh, B.H. (1985) Clin. Exp. Immunol. 60, 417–426.
[4] Kennett, R.H., Denis, K.A., Tung, A.S. and Klinman, N.R. (1978) Curr. Top. Microbiol. Immunol. 81, 77–91.
[5] Clark, M.R., Wright, B.W. and Milstein, C. (1981) Cited in Galfre, G. and Milstein, C., Methods Enzymol. 73, 3.
[6] Mann, D.M.A. and Sinclair, K.G.A. (1978) Neuropathol. Appl. Neurobiol. 4, 129–135.
[7] Puchtler, H., Sweat, F. and Levine, M. (1962) J. Histochem. Cytochem. 10, 355–364.
[8] Nandy, K. (1982) in: The Aging Brain (Hoyer, S. ed.) Exp. Brain Research, suppl.5, pp.123–126, Springer, Berlin.
[9] Wiśniewski, H.M., Bruce, M.E. and Fraser, H. (1975) Science 190, 1108–1110.