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HYBRIDOMA CELLS IN TUMOUR IMMUNITY

by

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ABSTRACT

Hybridoma cells have been used as targets to measure cytotoxic cells in a technique which uses the secretion of monoclonal antibody as an index of viability and a means of measuring the number of cells by the haemolytic plaque technique. The hybridoma has been shown to be susceptible to cytotoxic T cells, natural killer cells and cytotoxic cells arising spontaneously in culture.

From the original cloned hybridoma line, a number of variants have been isolated which grow as different types of tumours in syngeneic mice. One particular strain grows with high efficiency as tumours in the spleen. In contrast to the original hybridoma, the progressive spleen-seeking tumour cells appear to have lost their ability to stimulate the immune system.

The contribution that natural killer cell and T cell immunity plays in controlling tumour growth has been demonstrated and the *in vitro* analysis of immune effector cells in the spleen of mice challanged with tumour cells has been investigated.

These data illustrate the advantages of using hybridoma cells as a model to investigate tumour immunity.

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SUMMARY

1. The use of hybridoma cells as targets to measure cell-mediated cytotoxicity has been established. The ability of target hybridoma cells to form haemolytic plaques has been used as an indicator of target viability and therefore, the cytotoxicity has been detected by plaque reduction (PR). Allogeneic responses, anti-hapten responses, and spontaneous cytotoxic responses have been measured by the PR assay. Compared with the standard ^{51}Cr-release assay, a greatly enhanced sensitivity in detecting small amounts of cytotoxicity has been achieved by this PR technique. The advantage of using the PR assay lies in the ability to extend the assay time and to increase the effector to target cell ratio by using small numbers of target cells. (Chapter III - Part 1)

2. The PR technique has been further developed as a rapid screening assay, particularly suitable for limiting dilution analyses. Using hybridoma as targets, the cytotoxicity has been measured either by the loss of haemolytic plaque formation or by the reduction of the amount of haemolytic monoclonal antibody secreted from viable target cells into the assay supernatants. The assessment of large numbers of cytotoxic samples has been greatly facilitated by assaying the haemolytic antibody by measuring haemoglobin release in the assay with an automated micro-ELISA multi-scanner and by scoring visually using a modification of spot test. Using these new techniques, relatively high frequency estimates of CTL-precursors in an allogeneic response (1 in 462 spleen cells) and an anti-fluorescein response (1 in 3,970 spleen

cells) were obtained in primary 5 day limiting dilution cultures. (Chapter III - Part II)

3. Cytotoxic T cells which arise spontaneously in cultures (SCTL) have been characterized using a sensitive PR assay. Although SCTL effector cells were highly susceptible to anti-Thy-1 antibody plus complement treatment, they were-derived from precursor cells having very low levels of Thy-1 antigen. SCTL were barely detectable in cultures of thymus cells, bone marrow cells, and spleen cells from athymic nu/nu and very old mice. The target recognition pattern of SCTL did not follow the general rules of H-2 restriction and was clearly different from those of allogeneic CTL. (Chapter IV - Part I)

4. SCTL appeared to be kinetically and functionally distinct from natural killer (NK) cells. The specificity of these two types of cytotoxic cell populations was compared by both direct cytotoxicity tests and competitive target inhibition tests. The SCTL population consisted of an array of cytotoxic cells each of which was specific for a series of target cells. The NK cells had a more limited range of target selectivity, although at least two types of NK effector cells were detected on the basis of specificity measurements. (Chapter IV - Part II)

5. Progressive tumour variants have been selected from the regressive or non-tumourigenic original Abo-1 hybridoma by repeated passaging through syngeneic mice. A spleen-seeking variant (BSp) which metastasizes into spleens highly selec-

tively after 15 i.v. passages has been established and used as a tumour model to investigate tumour immunity. The tumourigenic nature of BSp has been characterized by comparing it with other cell lines and by following the survival of mice after a standard tumour cell challenge (10^5 cells i.v.). It has been determined that 10^5 cells is the minimum lethal dose of BSp tumour cells which can kill all syngeneic (BALB/c x DBA/2)F_1 mice within a period of 35-40 days after i.v. injection. When 10^5 BSp cells were injected i.v. into various strains of mice, a marked difference in the host survival and the incidence of paraplegia was observed. Even among syngeneic H-2^d strain of mice, BALB/c and (BALB/c x DBA/2)F_1 mice were susceptible to the tumour challenge, whereas DBA/2 mice were totally resistant. (Chapter V)

6. This tumour model has been further developed and established as a simple and reliable tumour growth assay *in vivo* to investigate the possible roles of immune constraints involved in tumour rejection. The growth of BSp cells can be measured by counting accurately the number of tumour haemolytic plaques in the spleen or by titrating the amount of haemolytic antibody in the serum without sacrificing the animals. Using this effective tumour growth assay, it was demonstrated that T cells and NK cells could play an important role in inhibiting the BSp tumour growth in non-immunized mice, and that immune T cells exhibited a strong anti-BSp anti-tumour response in the mice which had been immunized with the original Abo-1 cells. (Chapter V)

7. The difference in immunogenicity between the tumourigenic BSp cells and the original non-tumourigenic Abo-1 cells has been compared. It was confirmed that priming with Abo-1 cells could elicit strong T cell responses both in suppressing the tumour growth in vivo and in generating the anti-Abo-1 cytotoxicity in vitro. In contrast, BSp cells failed to induce such responses. It was concluded therefore that the loss of immunogenicity might be an important factor in the derivation of this progressive tumour. (Chapter VI)

8. During the generation of anti-Abo-1 CTL in cultures, not only the anti-Abo-1 cytotoxicity but also cytotoxicities against irrelevant other tumour targets have been generated. The anti-Abo-1 CTL responses are consistent with them being Abo-1 specific and H-2 restricted, however, these effector cells could recognize other syngeneic ($H-2^d$) tumour targets to some extent. The immunological significance of Abo-1 TAA has yet to be established but its potential interest and the implications of the findings are discussed with regard to tumour immunogenicity and immunotherapy. (Chapter VI)

ABBREVIATIONS

Ab	: antibody
AFC	: antibody forming cell(s)
Ag	: antigen(s)
AK	: anomalous killer
AR	: antibody reduction
BCG	: Bacillus Calmette-Guerin
C'	: complement
$C.D._{50}$	: median cytotoxic dose
Ci	: curie
CMI	: cell-mediated immunity
Con A	: concanavalin A
Cr	: chromium
CTL	: cytotoxic T lymphocytes
DMEM	: Dulbecco's modified Eagle's medium
DTH	: delayed type hypersensitivity
ELISA	: enzyme-linked immunosorbent assay
f	: frequency
FCS	: foetal calf serum
FDA	: fluorescein diacetate
FITC	: fluorescein isothiocyanate
g	: gram or gravity
h	: hour(s)
Hb	: haemoglobin
HS	: horse serum
IFN	: interferon
Ig G	: immunoglobulin G
IL	: interleukin (IL-1, IL-2 etc.)
i.p.	: intraperitoneally

i.v. : intravenouslly

l : litre

M : molar

m : milli (x 10^{-3}) or metre

2-ME : 2-mercaphoethanol

met+ : metastatic

MHC : major histocompatibility complex

min : minute(s)

Mls : M locus

n : nano (x 10^{-9})

Nc : national cytotoxic

NK : natural killer

O.D. : optical density

osm : osmolar

P : probability

PBS : phosphate buffered saline

PFC : plaque forming cell(s)

Poly I:C : polyinosinic-polycytidylic acid

PR : plaque reduction

r : coefficient of correlation

r^2 : coefficient of determination

RAFT : rat factor (supernatants of Con A-stimulated rat spleen cell cultures)

RBC : red blood cell(s)

SBSS : Shortman's balanced salt solution

s.c. : subcutaneously

SCTL : spontaneous cytotoxic T lymphocytes

ST-Hb : spot test - haemoglobin scan

ST-V : spot test - visual scan

TAA : tumour associated antigens

TATA	: tumour associated transplantation antigens
TCGF	: T cell growth factors
TNBS	: 2, 4, 6 - trinitrobenzene sulfonic acid
TNP	: 2, 4, 6 - trinitrophenyl
TSA	: tumour specific antigens
tum^+	: tumourigenic
UV	: ultraviolet (light)
v/v	: volume per volume

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