ANALYSIS OF CLONES OF CYTOTOXIC LYMPHOCYTES

by

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ABBREVIATIONS

DNA	deoxyribonucleic acid
Ci	curie
CI	cellular interaction
CL.P	cytotoxic lymphocyte precursors
CLs	cytotoxic lymphocytes
CMC	cell-mediated cytolysis
ConA	concanavalin A
Cr	chromium
cy	cyclophosphamide
DS	dextran sulphate
FCS	foetal calf serum
FITC	fluorescein isothiocyanate
g	gram
g	gravity
h	hour(s)
k	kilo, 10^{3}
l	litre
LPS	lipopolysaccharide B
M	molar
m	milli, 10^{-3}
2-ME	2-mercaptoethanol
MHC	major histocompatibility complex
min	minutes
minor H	minor histocompatibility
MLC	mixed lymphocyte cultures
MLTC	mixed lymphocyte-tumour cell cultures
MSV	moloney sarcoma virus

N	N-(3-nitro-4-hydroxy-5-iodophenylacetyl)-β-alanylglycylglycylglycyl
Pa	pascal
PBS	phosphate buffered saline
PHA	phytohaemagglutinin
POPOP	1,4-bis-(4-methyl-5 phenyloxazolyl)-benzene
POP	2,5-diphenyloxazole
μ	micro, 10^{-6}
TNBS	2,4,6-trinitrobenzene sulphonic acid
TNP	2,4,6-trinitrophenyl

SUMMARY

1. 'Spontaneously' generated cytotoxic clones were detected when normal spleen cells from CBA, DBA/2 or (CBA x DBA/2)F_1 mice were cultured in polyacrylamide cultures vessels without stimulator cells. Cytotoxicity was mediated by T cells and the highest number of clones occurred after 4 days in culture. The spontaneous cytotoxic T cell clones were detected mainly in adult spleen cell cultures. Few spontaneous clones were generated by lymph node, thymus or neonatal spleen cells.

2. The production of spontaneous clones does not increase linearly with the number of cells cultured which is in contrast with the production of 'stimulated' clones of cytotoxic lymphocytes in the polyacrylamide vessels. At the optimal cell concentration, 1.3×10^7 cells per culture, 20 spontaneous clones of CLs lysing P815 mastocytoma cell targets were detected in cultures of (CBA x DBA/2)F_1 spleen cells and 14 clones were detected in cultures of CBA or DBA/2 spleen cells.

3. The specificity of the spontaneous clones was examined by dividing each clone into two halves and assaying the half clones against a pair of different target cells. A range of spontaneous CLs of different specificities was produced. Spontaneous clones lysing syngeneic and allogeneic tumour or normal spleen cell blasts, as well as hapten-modified target cells were detected.

4. Individual spontaneous clones of CLs exhibited a high degree of discrimination and were able to differentiate between many pairs of target cells which were syngeneic with respect to each other.

5. When blast cells which had been induced by various mitogens were used as the target cells, the results indicated that spontaneous CL clones could discriminate between subsets of syngeneic lymphocytes which respond to different mitogens.

6. One-way stimulated CL responses were generated using cells from mice which had been treated with cyclophosphamide. Treatment of mice with 200 mg/kg cyclophosphamide abolished the ability of the cells to generate spontaneous clones in culture without impairing their ability to stimulate the production of CLs in responder cell populations.

7. In contrast with spontaneous clones which could discriminate between different $H2^d$ target cells, CLs produced by CBA spleen cells stimulated with $H2^d$ alloantigens were observed not to differentiate between various $H2^d$ target cells. The results indicated that spontaneous clones of CLs were not a representative sample of the stimulated clones of CLs.

8. When CBA spleen cells were stimulated simultaneously with $H2^b$ and $H2^d$ alloantigens, separate populations of CLs against the two sets of antigens were produced. Very few cross-reactive clones were detected.

9. When (CBA x $C_{57}Bl$)F_1 spleen cells were stimulated with syngeneic F_1 cells modified with TNP, clones of CLs lysing TNP modified cells of the F_1 and the two parental strains were produced. The frequency of clones produced by F_1 spleen cells against F_1-TNP, CBA-TNP and $C_{57}Bl$-TNP target cells was 1 per 3.3×10^4, 1 per 6.7×10^4, and 1 per 10^5 spleen cells respectively.

10. When (CBA x $C_{57}Bl$)F_1 cells were cultured with TNP-modified CBA cells, clones of CLs against TNP-modified cells of both the parental strains were produced. CLs which lyse CBA-TNP and CLs which lyse $C_{57}Bl$-TNP targets segregated as two distinct populations with no cross-reactivity.