

Lymphocytes

a practical approach

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Preface

Cellular immunology only came of age a mere 25 years ago, with the demonstration by James (now Sir James) Gowans and his coworkers that lymphocytes are the mediators of immunological responses. Until then the function of these numerous, widely distributed, but morphologically uninteresting cells was unknown. Even then it took some years before the full degree of heterogeneity of the cellular components of the lymphoid system became appreciated. This era yielded evidence for the division of labour between T and B cells, and the subsequent subdivision of these broad compartments into functional subpopulations of cells gradually emerged from the combined efforts of a growing body of immunologists. With this knowledge came the increasing awareness of the importance of cell-cell interactions in the initiation and control of immune responses, involving not only lymphocytes proper, but also a variety of accessory cells, which are needed to process and/or to present antigens to the actual protagonists in the immunological arena.

The desire to untangle the complex network of interacting and differentiating cells which are found in the sites of immune responses *in vivo* inevitably stimulated the development of novel methods. Thus, increasingly sophisticated procedures have evolved for the purification of lymphocyte and accessory cell subpopulations, together with culture and assay systems for measuring the growth and effector functions of both T and B cells *in vitro*. A major advance was the discovery that subpopulations of lymphoid cells can be distinguished by the array of marker molecules which they display on their surfaces, and the advent of monoclonal antibodies has markedly accelerated progress in this field.

This volume contains a collection of chapters dealing with various aspects of the study of lymphocytes *in vitro*. These cover methods for preparing lymphocytes and accessory cells from various sources, and for characterizing lymphocyte subpopulations, both in cell suspensions and in tissue sections. Other chapters deal with techniques for culturing and cloning lymphocytes and for measuring their differentiated effector functions, whether these be antibody secretion, or the capacity to kill virus-infected target cells. Most of the volume concerns the biology of lymphocytes. However, enormous strides have also been made in understanding the molecular biology of these cells. It therefore seemed appropriate to also provide the reader with a flavour of some of the contemporary methods being used for characterizing the surface molecules which lymphocytes use for communicating with their environment.

Although the last 25 years have produced an impressive body of knowledge about these fascinating cells, it is clear that many questions about the induction and regulation of immune responses remain unanswered. Hopefully this volume will provide a useful compendium of procedures which can be applied to answering at least some of these questions.

Gerry G.B.Klaus

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Abbreviations

AET	2-amino ethylisothiuronium bromide hydrobromide
AIDS	acquired immunodeficiency syndrome
ALL	acute lymphocytic leukaemia
AML	acute myeloid leukaemia
AMP	2-amino-2 methyl-1-propanol
AO	acridine orange
AP	alkaline phosphatase
APAAP	immuno-alkaline phosphatase-anti-alkaline phosphatase
APC	antigen-presenting cells
ARS	AIDS-related syndromes
ATCC	American Type Culture Collection
BCDF	B cell differentiation factor
BCGF	B cell growth factor
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BHK	baby hamster kidney
BL	Burkitt's lymphoma
BLCL	B lymphoblastoid cell lines
BrdU	bromodeoxyuridine
BSA	bovine serum albumin
BSE	biotin succinimide ester
BSF	B cell-stimulatory factor
BSS	balanced salt solution
CD	Cluster of differentiation
CFT	complement fixation test
CML	cell-mediated lympholysis
CLL	chronic lymphocytic leukaemia
Con A	concanavalin A
CSF	colony-stimulating factor
CTL	cytotoxic T cells
DAB	3,3'-diaminobenzidine
DABCO	diazo-bicyclooctane
DMSO	dimethyl sulphoxide
DNP	2,4-dinitrophenyl
DTT	dithiothreitol
EB	ethidium bromide
EBNA	Epstein-Barr virus nuclear antigen
EBV	Epstein-Barr virus
EDF	eosinophil differentiation factor
EDTA	ethylene diamine tetraacetic acetate
ELISA	enzyme-linked immunosorbent assay
Endo F	endo- β -N-acetylglucosaminidase F
Endo H	endo- β -N-acetylglucosaminidase H
EP	endogenous pyrogen
FACS	fluorescence-activated cell sorter
FCS	fetal calf serum
FDC	follicular dendritic cell(s)
FITC	fluorescein isothiocyanate

HBSS	Hank's balanced salt solution
Hepes	<i>N</i> -2-hydroxyethyl piperazine- <i>N'</i> -2-ethane sulphonic acid
HEV	high endothelial venules
HLA	human leucocyte antigen
HPGF	hybridoma/plasmacytoma growth factor
HRP	horseradish peroxidase
IEF	isoelectric focusing
IF	immunofluorescence
IHC	immunohistochemical
IL	interleukin
KLH	keyhole limpet haemocyanin
LAK	lymphokine-activated killer
LDA	limiting dilution analysis
LEM	leukocyte endogenous mediator
LGL	large granular lymphocyte
LPS	(bacterial) lipopolysaccharide
MAb	monoclonal antibody
MCGF	mast cell growth factor
MEM	minimal essential medium
MHC	major histocompatibility complex
MLR	mixed leukocyte reaction
Mops	3-(<i>N</i> -morpholino)propanesulphonic acid
NEPHGE	non-equilibrium pH gradient electrophoresis
NIP	nitro-iodo-phenacetyl
NK	natural killer
NP-40	Nonidet P-40
OPD	<i>o</i> -phenylenediamine
ORBC	ox red blood cells
PBL	peripheral blood lymphocytes
PBMC	peripheral blood mononuclear cells
PBS	phosphate-buffered saline
PE	phycoerythrin
PEC	peritoneal exudate cells
PFC	plaque-forming cell(s)
PHA	phytohaemagglutinin
PLL	poly-L-lysine
PMA	phorbol myristic acetate
PMSF	phenylmethylsulphonyl fluoride
POL	polymerized flagellin (from <i>Salmonella</i> spp.)
PPD	purified protein derivative
PWM	pokeweed mitogen
RBC	red blood cells
RIA	radioimmunoassay
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SKSD	streptokinase/streptodornase
SRBC	sheep red blood cells
Tc	cytotoxic T cell
T _h	helper T cell
TBS	Tris-buffered saline
TCA	trichloroacetic acid

TCGF	T cell growth factor
TRF	T cell replacing factor
TDL	thoracic duct lymphocytes
TdT	terminal deoxynucleotidyl transferase
TG	thioglycollate
TEMED	<i>N,N,N',N'</i> -tetramethylethylenediamine
TNBS	trinitrobenzyl sulphonic acid
TNP	2,4,6-trinitrophenyl
TPA	12- <i>O</i> -tetradecanoyl phorbol-13-acetate
TRITC	tetramethylrhodamine isothiocyanate
VDU	visual display unit
ZIPP	zones of inhibited phage plaques

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