PEDIATRICS UPDATE

Reviews for Physicians

1984 Edition

Arthur J. Moss, M.D.
Editor-in-Chief

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PREFACE

This volume of *Pediatrics Update: Reviews for Physicians* is comprised of contributions from 29 experts in their respective fields. Again, this edition is designed to keep the reader abreast of recent developments in several key areas of pediatrics. It should be of interest and of value to pediatricians, family practitioners, internists, house officers, and students.

The topics cover a wide age span ranging from the fetus to the adolescent. Likewise, the range of subjects covered is wide. Of particular interest in this volume is the application of monoclonal antibodies in the diagnosis and treatment of childhood cancer. Concerning the neonate, nutrition, toxoplasmosis, bronchopulmonary dysplasia, intracranial hemorrhage, heart disease in infants of diabetic mothers, and the predictive value of neurologic abnormalities are discussed. In the adolescent, the physiology of puberty, eating disorders, and goiter are dealt with.

Two chapters, generously supplemented with illustrations, focus on the rapidly emerging discipline of ultrasound. Other subjects, which will no doubt be of interest to the reader, include multiple sclerosis, gastroesophageal reflux, Reye syndrome, herpesviruses, edema in infancy and childhood, attention deficit disorders, and children's understanding of illness.

The broad spectrum of topics should be of value in practice, in keeping pace with the scientific literature, and in reviewing for board certification or recertification.

The editors appreciate comments from the readers including suggestions of subjects for subsequent articles.

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CONTENTS

MONOCLONAL ANTIBODIES IN THE DIAGNOSIS AND TREATMENT OF CHILDHOOD CANCER

Adrienne C. Altman, M.D.
and Robert C. Seeger, M.D.

The potential contributions of immunology to the diagnosis and treatment of cancer have long been recognized. The specific nature of immune responses suggests that they may provide the means to distinguish neoplastic from normal cells. In the area of immunodiagnosis, this potential has begun to be realized. However, the success of immunotherapy has been limited. Indeed, it has become clear that tumors, the immune system, and immune system–tumor interactions are highly complex and that considerable fundamental knowledge is necessary if the potential benefits of immunotherapy are to be fully realized.

Recent advances in immunology have made it possible to generate and maintain individual clones of antibody-secreting hybrid lymphocytes termed lymphocyte hybridomas. The ability of these clones to produce a single type of antibody, which is therefore called a monoclonal antibody, has revolutionized serology, and there is little doubt that this

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technology will make significant contributions to the diagnosis and treatment of cancer as well as many other diseases. This chapter discusses the principles of monoclonal antibody production and the potential clinical usefulness of such reagents based on their ability to react with tumor cell surface molecules. Childhood acute lymphocytic leukemia (ALL) and neuroblastoma, the most studied leukemia and solid tumor, respectively, are reviewed with regard to the impact of monoclonal antibodies on the diagnosis and therapy of childhood neoplasms.

MONOCLONAL ANTIBODIES

In 1975, Kohler and Milstein[40] demonstrated that immortal clones of antibody-producing cells could be generated by cell fusion in which a normal B lymphocyte was fused with a myeloma cell. The B lymphocyte provides the genetic information for antibody production, whereas the myeloma cell provides the machinery and immortality. Once such cells are fused and then cloned, a continuously growing cell line producing an immunoglobulin that recognizes a single antigenic determinant is obtained. This immunoglobulin can be obtained in pure form and in essentially unlimited quantities. The steps involved in making lymphocyte hybridomas are illustrated in Table 1. Although nearly all monoclonal antibodies produced to date have been murine, some have been human. The latter clearly will be of importance for therapy. The subject of monoclonal antibodies has been completely reviewed in three recent publications.[36,47,52]

TUMOR CELL SURFACE ANTIGENS

Principles

The tumor cell surface is a major focus for immune responses. Molecular determinants of cell surface proteins, glycoproteins, lipids, phospholipids, and glycolipids that elicit immune responses are termed cell surface antigens. Most such antigens identified on neoplastic cells also are expressed by some normal cells (Table 2). The functions of nearly all these components, with the exception of those associated with some receptors, are unknown. Cell surface determinants that are relatively selectively expressed by tumor cells may be operationally tumor-specific under defined circumstances and thus may serve as markers for immunodiagnosis and as targets for immunotherapy. These include antigens on receptors, histocompatibility molecules, and differentiation molecules. Differentiation antigens are selectively expressed by normal cells according to their cell lineage and stage of development.

Tumor-specific antigens, by the strictest definition, are not present on the subject's normal cells during any stage of development. To date, there is no compelling evidence that they exist among human neoplastic cells. It is difficult to define these antigens with certainty because normal cells comparable to tumor cells with respect to cell lineage and degree of differentiation must be identified and analyzed. Furthermore, most

TABLE 1. Production and Characterization of Monoclonal Antibodies

1. Immunize mouse with antigen preparation to generate immune B lymphocytes.
2. Fuse immune B lymphocytes from mouse spleen with continuously dividing murine myeloma cells to obtain B-lymphocyte–myeloma hybrid cells. These cells are termed *lymphocyte hybridomas*.
3. Grow lymphocyte hybridomas in vitro in culture medium that allows them but not parental myeloma cells to survive. Parental immune B lymphocytes are not capable of continuous growth in vitro. Therefore, the only cells that survive are lymphocyte hybridomas.
4. Identify lymphocyte hybridomas that produce potentially useful antibodies.
5. Clone antibody-producing lymphocyte hybridomas to ensure that the antibody is being produced by one clone of cells. An antibody produced by a single clone of lymphocyte hybridoma cells is termed a *monoclonal antibody*.
6. Identify cells that react with the monoclonal antibody and characterize the antigenic molecule biochemically.

newly recognized tumor cell surface molecules are likely to be previously undefined products of normal genes rather than of tumor-specific genes, since only about 10^3 gene products have been identified for the approximately 10^6 genes in mammalian cells.[13] For practical purposes, however, tumor specificity is not necessary if an antigen is useful.

Malignancies, although monoclonal in origin, consist of a population of neoplastic cells that may be heterogeneous in a number of ways. Expression of cell surface components and of tumor cell products may vary. Such heterogeneity would be a major consideration with regard to immune response, immunodiagnosis, and immunotherapy. The therapeutic process itself may promote changes in surface character either directly, e.g., antigenic modulation,[61] or indirectly by affecting the cell cycle or differentiation. Phenotypic variation in surface marker expression has been demonstrated among ALL cells with respect to binding of sheep erythrocytes,[48] expression of common ALL antigen (CALLA),[48,64] and monoclonal antibody-defined determinants.[70] Differences may exist at a single time or in different stages of active disease.[3,48] Although individual neuroblastomas have not been studied for immunological heterogeneity, there is good reason to suspect that it exists. Neuroblastoma cells can be in various stages of the cell cycle, can be noncycling, and can be differentiated to varying degrees.[14,26,75]

TABLE 2. Types and Examples of Human Tumor Cell Surface Antigens

1. Antigens associated with receptors: sheep erythrocyte and transferrin receptors.
2. Histocompatibility antigens: HLA-A, B, and DR (Ia-like)
3. Blood group antigens: P determinant, M and N precursor
4. Differentiation antigens
   a. Cell lineage-related: surface immunoglobulin of B lymphocytes
   b. Maturational stage-related: Thy-1 of myoblasts, thymocytes, neurons, glia
5. Virus-associated antigens: viral capsid antigen of Epstein-Barr virus
6. Tumor-specific antigens: not unequivocally defined for human tumors

Lymphocytic Leukemia

Leukemic cells variously display many of the known surface markers of their nonmalignant counterparts (B, T, null, and immature lymphoid cells). These markers include some with recognized functions such as the receptors for the Fc portion of immunoglobulin or for complement, and the surface immunoglobulin that acts as an antigen receptor for the B cell. Histocompatibility antigens HLA-A, B, C, and DR (Ia-like) can be expressed. Other markers serve no known purpose but have been useful in subclassifying normal lymphocytes, and some carry prognostic significance for the corresponding leukemia. Receptors for sheep erythrocytes (SRBC or E receptors) on T cells cause them to form rosettes with the red blood cells in vitro. The most frequent type of ALL characteristically expresses CALLA. Null ALL cells lack these markers. Table 3 indicates the conventional surface marker classification of ALL.

Many monoclonal antibodies to leukemic cells have been generated, as reviewed recently by Nadler et al.[54] Some of these antibodies detect receptors associated with lymphoid cell function, histocompatibility structures, and normal differentiation antigens. The more precise subclassification of leukemias possible with monoclonal antibodies should prove useful in the study of nonmalignant lymphoid differentiation as well as allow better prognostication and tailoring of therapy. Table 4 lists some monoclonal antibodies that have been developed.

Neuroblastoma

Human neuroblastoma cells can express receptors for various molecules including transferrin and insulin, which regulate cell proliferation,[7] and nerve growth factor, which regulates proliferation and differentiation.[53] Although human neuroblastoma cells have not been evaluated for neurotransmitter and neuropeptide receptors, different clones of murine C1300 neuroblastoma cells have been shown to have acetylcholine, β-adrenergic, dopamine, substance P, γ-aminobutyric acid, opiate, adenosine, prostaglandin, and histamine H_1 receptors.[7] Some of

TABLE 3. Conventional Lymphoid Markers Defining Subtypes of ALL

Cell marker	ALL subtype					
	Common	Pre-B	B	Pre-T[a]	T	Null
CALLA	+	+	−	−	−[b]	−
Ia-like antigen	+	+	+	−	−	+/−
Immunoglobulin						
Cytoplasmic μ chain only (no light chain)	−	+	−	−	−	−
Complete cytoplasmic or surface Ig	−	−	+	−	−	−
SRBC receptor (rosette formation)	−	−	−	−	+	−

[a]Pre-T-ALL cannot be defined with these markers but can be recognized with monoclonal antibodies.

[b]CALLA is expressed by leukemic cells from 10% of T-ALL patients.

TABLE 4. Markers Associated with Subtypes of ALL That Are Defined by Monoclonal Antibodies

Cell category	Monoclonal antibody	Structure[a]	ALL subtype						Ref.
			Common	Pre-B	B	Pre-T	T	Null	
Progenitor cell	J-5, BA-3	CALLA, gp100	+	+	−	−	−	−	42,56,59
	BA-2	p24	+				±		37
B-cell lineage	B1		+	+	+	−	−	−	54
	BA-1		+	+	+	−	−	−	1
	7.2	Ia, gp28,34	+	+	+	−	−	−	25
T-cell lineage	Leu-1, OKT1, T101	gp65	−	−	−	+	+	−	16,66
	OKT3	gp19	−	−	−	−	+	−	54
	9.6, OKT11	SRBC rec., gp45	−	−	−	−	+	−	25,29

[a]gp, Glycoprotein; p, protein; gp100, glycoprotein with a relative molecular weight of 100,000; Ia, Ia-like molecule; SRBC rec., sheep red blood cell receptor.

these receptors are selectively expressed by neuroblastoma cells compared to most normal cells, and antibodies reacting with those required for proliferation may be particularly effective immunotherapeutically since elimination of receptor-positive cells would eradicate tumor cell proliferation.

Major histocompatibility antigens of the *HLA-A* and *B* loci with the same specificity as those on autologous normal cells have been demonstrated on neuroblastoma cells.[44] Additional HLA-like molecules not present on normal host cells also have been reported[44]; it will be important to confirm their presence and determine their identity since, as foreign antigens, they could evoke antitumor responses. Indeed, histocompatibility antigens have been demonstrated on murine tumor cells that are not on normal autologous cells and are capable of eliciting effective antitumor immune responses.[19] Human Ia-like antigens are also part of the major histocompatibility complex. Although they are present on most melanoma cells, they have not been detected on neuroblastoma cells.[28]

A number of antigens characteristic of normal nervous system cells (neural cells) have been defined on neuroblastoma cells with monoclonal antibodies (Table 5). Some of these antigens appear to be limited to fetal neural tissues or to be present in a greater quantity in fetal than in adult tissues. Presumably, such antigens are characteristic of immature cells. Other differentiation antigens are expressed by both fetal and adult neural tissues or primarily by adult tissues.

Thy-1 is an example of a cell surface differentiation molecule expressed by human neuroblastoma cells[72,73] (Table 5). Among normal cells, Thy-1 is expressed by adult brain in at least 10-fold greater quantity than by fetal brain,[73] by neurons and glia,[32] by myoblasts,[77] by fibroblasts,[46,73,77] by a subpopulation of thymocytes,[46] and probably by a subpopulation of kidney cells.[73] Neuroblastoma, glioma, rhabdomyo-