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Monoclonal Antibodies to Human T Lymphocyte Surface Antigens: Their Potential Application to Urology

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Introduction

The advent of monoclonal antibodies has been one of those rare innovations which has potential application to almost every branch of medical science. The application of this innovation has now spread well beyond the research laboratory into the field of diagnosis and therapy.

The review describes the principles underlying the production, characterisation and application of monoclonal antibodies with particular reference to the identification of lymphocyte sub-populations. The applications of this technology to urology and renal transplantation are then discussed.

This field of research is growing fast and this article is not comprehensive but rather aims to be an introduction for the clinician to this important topic.

Outline of Cellular Immunology

At the centre of the study of both cellular and humoral immunology is the lymphocyte. Although lymphocytes are morphologically similar when studied with the light microscope, they are functionally heterogeneous. The identification of sub-populations within the lymphocyte series was possible before the advent of monoclonal antibodies, through the detection of a variety of cell surface markers. These included products of the major histocompatability complex (M.H.C.) which occur as cell surface proteins, which can be defined by biochemical and immunological methods.

In addition, there are cell membrane receptors for a variety of biological structures such as lectins, sheep erythrocytes and complement components. Using these surface markers one can define two major lymphocyte sub-populations, namely T and B cells. Cells of both these sub-populations are derived from stem cells in the bone marrow. The T cells are so-called because of their subsequent migration to and maturation in the thymus. B lymphocytes are defined as cells which are synthesising immunoglobulin. At the end of the B cell lineage is the plasma cell, which secretes immunoglobulin.

The use of monoclonal antibodies has allowed the process of classification to go further by identifying numerous sub-populations of the lymphomyeloid series. This article is concerned with identification of functional sub-populations of mature T lymphocytes.

Before describing these sub-populations and their interactions in the immune response, a description of the production of monoclonal antibodies to lymphocyte surface antigens will be given.

Monoclonal Antibody Production

Burnet's theory of clonal selection [1] stated that immunoglobulin of only one specificity would be produced by each individual plasma cell. While this theory has long been accepted, its validity was convincingly demonstrated experimentally when Kohler and Milstein [14] reported that hybrid cells secreting immunoglobulin which was homogeneous with respect to specificity, idiotype, allotype and class (i.e. monoclonal) could be obtained by fusing spleen cells from an immunised animal with certain myeloma cells. In the present review the principles of monoclonal antibody production will be illustrated by reference to the production of antibodies to lymphocyte cell surface antigens. However, the principles outlined are applicable to any substance that has antigenic properties in the immunised species. Most monoclonal antibodies described to date have been produced by fusing rodent lymphoid cells but there are a number of reports on antibodies produced by human-human hybrids [4, 29]. The basic steps employed are illustrated in Fig. 1 and summarised below.

Initially the mouse is immunised by the intraperitoneal or intravenous injection of purified T lymphocytes and at an appropriate time the spleen is removed from the mouse and a cell suspension made. The cells are then mixed with

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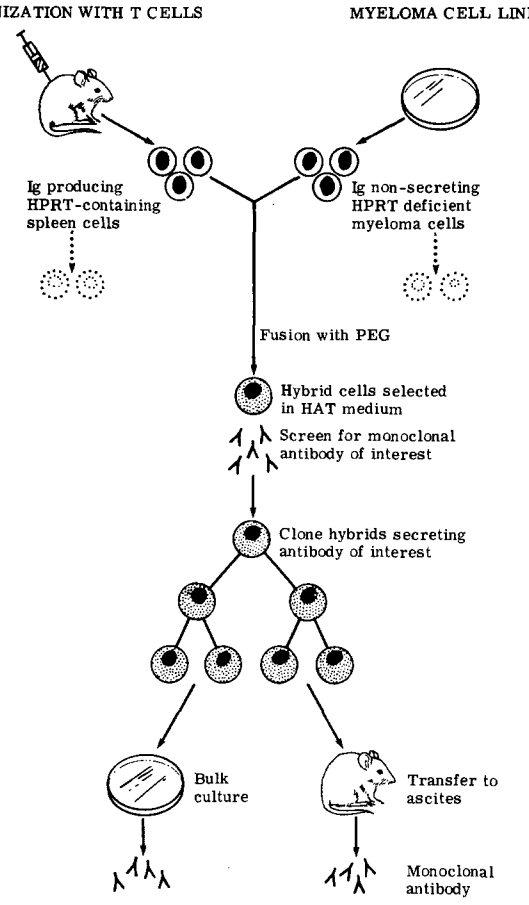

Fig. 1. *Monoclonal Antibody Production Ig* = Immunoglobulin; *HPRT* = Hypoxanthine phosphoribosyl transferase; *PEG* = Polyethylene glycol; *HAT medium* = Medium containing hypoxanthine, aminopterin and thymidine

those of a non-secreting myeloma cell line and cell fusion is performed by adding polyethylene glycol (P.E.G.) or inactivated Sendai virus. The resulting hybrid cells are few in number in relation to the parent populations and have to be selected from the unfused cells. This is done by using myeloma cell lines that lack the enzyme hypoxanthine phosphoribosyl transferase (H.P.R.T.). After fusion, the cell mixture (hybrids + unfused parent cells) is grown in medium containing hypoxanthine, aminopterin and thymidine. The unfused myeloma cells are unable to use the hypoxanthine for purine synthesis; they switch to endogenous synthesis, but this is blocked by the aminopterin and thus the cells die. The hybrid cells, by contrast, possess the appropriate genetic material for synthesis of hypoxanthine phosphoribosyl transferase (this being derived from the normal spleen cells) and survive. The unfused spleen cells succumb after a period of days on account of lymphocyte growth factor deficiency.

The hybrids which will be of interest are those resulting from fusion of a single spleen cell producing antibody to a lymphocyte surface antigen with a single myeloma cell. The fact that this occurs has been confirmed by biochemical studies [14]. Antibody secreted by such cells is monoclonal and provided that the hybrid cells maintain their chromosomal complement, an immortal production line of antigen specific antibody can be created.

In practice the hybrids of interest form a tiny minority of the hybrids created by the fusion and all the hybrids have to be screened for antibody production before those of interest are cloned and amplified.

It is of great importance to have an efficient system for screening the hybrids and the refinement of solid phase radioimmunoassay and the development of enzyme linked immunoassay has been of great help in the production of these antibodies. The production of specific antibody to T cell surface antigens can also be detected by an indirect immunofluorescence assay. If the monoclonal antibody is produced in a mouse then a fluorescein-conjugated goat anti-mouse antibody will bind to those cells binding the mouse monoclonal. These can then be identified and enumerated using a flow cytometer (Fluorescence Activated Cell Sorter [10] or Cytofluorograf [11]).

Having selected the hybrids which are secreting antibody of interest, the cells must be cloned and then either grown in bulk culture or maintained in ascites form – that is injected into the peritoneal cavity of a mouse where they grow and produce ascites which is rich in immunoglobulin.

The monoclonal antibody thus produced and the sub-population of cells bearing the surface antigen which it recognises, must now be further characterised. In the case of T cell sub-populations different groups [7, 12, 15, 23, 27] have found a basic triad of antibodies reacting with peripheral blood T cells in normal subjects. One of these reacts with approximately 95% of cells that form E rosettes, another reacts with approximately 60% of such cells, while a third reacts with approximately 30% of such cells. It should be noted that this discussion is confined to mature circulating lymphoid cells.

Other monoclonal antibodies have been of great value in the orderly dissection of the process of differentiation and maturation of these cells in bone marrow and thymus [20].

The characterisation of the sub-populations of mature T cells recognized by monoclonal antibodies has been performed by comparison with established cell surface markers, study of the anatomical localisation of the cells in the tissues of the lymphoid system and analysis of their function and interactions in the immune response.

Functional Studies

The main function of T lymphocytes is to regulate (help or suppress) the immune response to antigen. In addition, they have a role in the recognition of specific antigen and the execution of specific effector functions. In vitro, T cells respond to soluble and cell surface antigens and polyclonal activators including the lectin mitogens concanavalin A (Con A) and phytohaemagglutinin (PHA). They also

Table 1. Response of T cell subsets to in vitro stimulation

Stimulus	Helper/ Inducer Subset	Suppressor/ Cytotoxic Subset
Response to soluble antigen	+	–
Response to cell surface antigen	+	+
Response to PHA	+	Minimal
Response to Con A	+	+
Cytotoxicity after allosensitization	–	+

PHA = Phytohaemagglutinin; Con A = Concanavalin A

produce soluble factors which effect the differentiation and function of other lymphoreticular cells such as macrophages and granulocytes. Specific effector functions include the production of target cell lysis.

Studies in man with monoclonal antibodies have identified two major subsets within the T cell population. These subsets have been defined on the basis of the above in vitro functional tests. The two subsets are the so called helper/inducer subset and the suppressor/cytotoxic subset. Table I lists the response of these subsets to in vitro tests.

In addition to the in vitro effects described above it is now apparent that certain T cell subsets may influence the interactions of B lymphocytes, macrophages and of each other ie so called T-T interactions [21]. For example, if the helper/inducer cells are separated and then mixed with B cells from the same subject, mitosis is induced in the B cells which differentiate into plasma cells. The isolated suppressor/cytotoxic subset has no such action [19]. T-T interactions are demonstrated by the fact that although the helper/inducer subset is not cytotoxic per se, it is required for optimal development of cytotoxicity by the suppressor/cytotoxic population. Many other examples of different functions of these two subsets exist and point to their role in a delicate homeostasis of the immune respones (Fig. 2).

As the names of the helper/inducer and suppressor/cytotoxic subsets imply, there is likely to be heterogeneity within these populations. Evidence for heterogeneity within the helper/inducer subset comes from the observation that activation of this subset with soluble antigen produces a proportion of cells that express Ia-like antigens on their surface, that is antigens coded for by the I region of the major histocompatability complex. After isolation only those cells of the helper/inducer subset expressing Ia-like antigen produced non-specific helper factors [22].

There seems little doubt that the process of subclassification on the basis of surface markers and functional tests will continue. The examples given here provide a clear picture of the logical process of subset dissection that has been facilitated by the use of monoclonal antibodies.

Tissue Distribution of Subsets

The study of the anatomical localisation and traffic of constituents cells of the lymphoid system has also been facilitated by monoclonal antibodies. Using frozen tissue sections it is possible to stain selectively cells bearing various surface markers.

Sections cut from fresh frozen tissue are lightly fixed in acetone and then incubated with monoclonal antibody. A peroxidase conjugated rabbit or goat anti-mouse (the monoclonal antibody being of mouse origin) is then applied as a second layer. The peroxidase stain can then be visualised using diaminobenzidine or aminoethyl carbazole. Using this technique it has been possible to show that most lymph node T cells are of the helper/inducer subset and these cells are to be found in the germinal centres. Thus it can be shown that T and B cells are in anatomical juxta-position permitting contact between antibody-producing cells and antigen-primed cells [18]. The technique has also proved

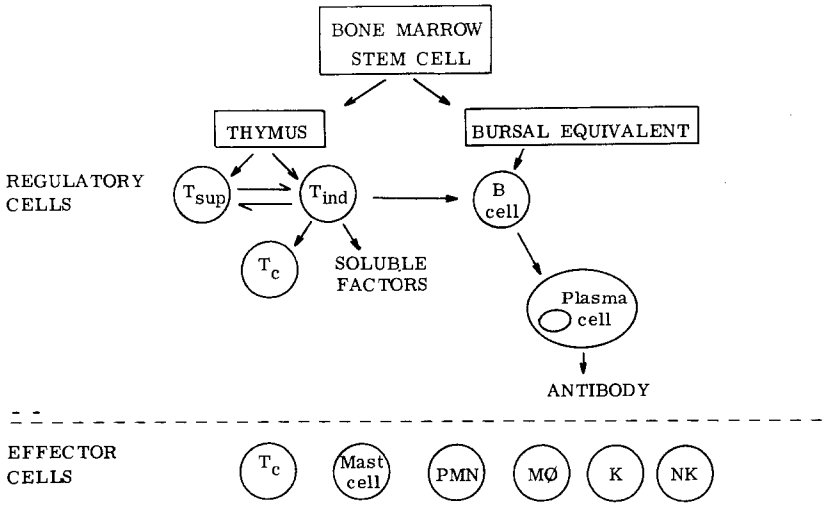

Fig. 2. *Simplified Outline of Cellular Interactions in the Immune Response. Tc* = Cytotoxic T cell; *PMN* = Polymorphonuclear leucocyte; *MØ* = Macrophage; *K* = Antibody dependent killer cell; *NK* = Natural killer cell; T_{sup} = Suppressor T cell; T_{ind} = Inducer/helper T cell. Based on Reinherz and Schlossman [22]

Table 2. Commercially available monoclonal antibodies and the subpopulations they recognise

Lymphocyte Subpopulation	Monoclonal Antibody Designation	Approximate % of Reactive Cells
All peripheral T cells	Leu1, Leu4, OKT3 T11	70
Helper/inducer T cells	Leu3a, Leu3b, OKT4 T4	43
Suppressor/cytotoxic T cells	Leu2a, Leu2b, OKT8 T8	20
B cells	HLA-Dr, B1	17

Leu – series marketed by Becton Dickinson; OKT – series marketed by Ortho Pharmaceuticals; T – series marketed by Coulter Electronics Inc.

of value in the study of lymphoid tumours and has potential application to the study of lymphoid infiltrates in solid tumours. The immunoperoxidase techniques involved have recently been reviewed by Farr and Nakane [8].

Study of subset function and localisation contribute to a picture of immune homeostasis resulting from a delicate balance of helper and suppressor activity on immunoglobulin production, macrophage activation and haemopoietic differentiation. Disturbance of this balance may result in a variety of immunopathological disorders. Immunodeficiency may result from loss of the helper/inducer subset or activation of the suppressor cell population.

The study of subset imbalance in a wide variety of disease states, including SLE, rheumatoid arthritis, infectious mononucleosis, myasthenia gravis, graft versus host disease, mycoses fungoides and leprosy have been reported [21] and many more diseases are under study.

Such studies are likely to increase dramatically in the near future as monoclonal antibodies to human lymphocyte differentiation antigens are now available from a number of commercial sources. Table 2 lists some of the companies marketing well-characterised reagents.

The Identification, Enumeration and Isolation of Cells Within a Subset

Mononuclear cells are usually isolated from peripheral blood by density gradient centrifugation, before incubation with the antibodies. However, buffy coat or whole blood preparations can be used [11]. After incubation of a cell population with a particular monoclonal antibody a method is required to identify, enumerate and if necessary isolate the cells binding the antibody. A number of techniques have been developed.

Cytotoxicity Assays

By incubating cells with antibody and complement, lysis of the cells binding the antibody will occur. Counting of the dead cells (i.e. those failing to exclude vital dyes) enables one to estimate the percentage of cells binding the antibody.

Limitations to this technique include the fact that not all subclasses of immunoglobulin bind complement and careful controls are required to ensure that the animal serum, used as a source of complement, is not itself cytotoxic.

Rosette Tests

Red cells from sheep or oxen can be coated with monoclonal antibody [16] and the formation of rosettes with lymphoid cell suspension used to enumerate cells bearing the relevant surface antigen.

Solid Phase Selection

The application of cell suspensions to antibody coated petri dishes permits the positive and negative selection of subsets [9]. The cells may also be selected on columns consisting of the appropriate antibody linked to a suitable matrix, e.g. sepharose.

Direct and Indirect Immunofluorescence

Monoclonal antibody can be directly conjugated with fluorescein isothiocyanate (green fluorescence) or tetramethylrhodamine isothiocyanate (red fluorescence). Alternatively a second stage antibody conjugate raised in a different animal against the monoclonal species can be used. Cells can then be positively identified by virtue of their cell membrane fluorescence, using fluorescence microscopy or flow cytometry. Flow cytometry has proved an invaluable technique as very large numbers of cells can be rapidly analysed. Examples of instruments for such use are the fluorescence Activated Cell Sorter (FACS IV [10] Becton Dickinson), the Electronically Programmable Individual Cell Sorter (EPICS V Coulter Electronics Incorporated) and the Cytofluorograf [11], (Ortho Instruments). Using this equipment one can also separate different cell populations for use in further functional studies.

In summary the methods of production of monoclonal antibodies to T cell subsets have been described, the characterisation and function of the subsets have been detailed and the methods of subset identification have been outlined. What then is the application and potential for these elegant reagents in the field of urology and transplantation surgery?

Clinical Applications

As previously noted, abnormalities of T cell subsets have been identified in a number of disease states [21]. These studies have been of importance in contributing to the concept of the helper-suppressor ratio as an important parameter of immunoregulatory status. The present review however, is limited to conditions of particular urological interest.

Renal Allograft Transplantation

Recent reports of serial estimation of lymphocyte subsets following renal transplantation [3, 6] have aroused considerable interest. These studies have demonstrated significant alterations in the ratio of helper/inducer to suppressor/cytotoxic cells in some patients following transplantation. Those patients whose ratios remained at the preoperative level (i.e. with an excess of the helper/inducer subset) seemed to be at risk of rejection, unless the donor was HLA identical or the total number of T cells was extremely low. These findings will require confirmation and large numbers of patients will be required for such studies; the patients will require to be stratified according to a number of variables, including the type of immunosuppressive regime used, the effect of post operative infection, the HLA cross-match, the type of rejection encountered and the blood transfusion history of the patient.

If the correlation of helper-suppressor ratios in these complex clinical states is confirmed, the monitoring of patients in this way may well be of great help to the clinician in the diagnosis of rejection and the individual tailoring of immunosuppressive therapy.

In addition to the use of monoclonal antibodies for such diagnostic purposes, significant prolongation of monkey kidney allografts after treatment with OKT 4 (anti helper/inducer subset) antibody was been reported [2]. This has been followed up by successful immunosuppression using OKT 3 (anti mature peripheral T cells) antibody in two human allograft recipients [3]. Hypersensitivity to repeated injection of mouse immunoglobulin did not seem to be a problem in these two patients but this is a potential drawback to this form of therapy. Technical problems apart, this therapeutic approach is an exciting development and these two reports demonstrate that selective subset manipulation is possible.

In addition to peripheral blood studies, quantitative analysis of interstitial cells in grafts undergoing rejection reveals that most infiltrating cells are T cells and the greater proportion are OKT 8 positive (suppressor/cytotoxic subset) [17]. This suggests and important role for the cells defined by this antibody (OKT 8) in the process of graft rejection by the cell mediated limb of the immune response.

Tumour Immunology

The disappointing results of immunotherapy over the past decade underline our incomplete understanding of the disorders of immunoregulatory function in malignant disease. The developments in technology previously described offer great potential in the further study of the tumour host relationship.

Enumeration of relative proportions of subsets, absolute numbers and functional studies on isolated subsets can be correlated with disease stage in cross sectional studies but perhaps of greater value, longitudinal studies will allow correlation with disease progression and various treatment modalities.

Immunological aspects of renal carcinoma, for example, have been reviewed by de Kernion et al. [5], who point to numerous studies documenting defects of cellular and humoral immune responses. In the light of these findings, the report of an improved response rate in 49 patients with metastatic renal carcinoma treated by preoperative arterial embolisation, nephrectomy and hormonal therapy [28], is of interest. Since there is little evidence to suggest that medroxyprogesterone acetate, used at the dose described, would produce this response, the suggestion was made that the improved response rate may be due to "an ischaemia-related immunological change". Well-controlled longitudinal studies of lymphocyte subsets may cast some light on the host response to embolisation and nephrectomy in this disease.

Mononuclear cell infiltration of tumours including those presenting to urologists have received considerable study. Kaszubowski et al. [13] in a study of tumour tissue from 46 patients with a variety of tumours, have shown that tumour lymphocyte infiltrates were composed mainly of T cells. A high proportion of these cells were Ia positive suggesting functional activation. Using the peroxidase or fluorescein labelled double antibody techniques previously described it should now be possible to determine the functional subtype of these intra-tumour T lymphocytes. If correlation between peripheral blood and intra-tumour subset changes can be demonstrated, and if these analyses suggest a consistent defect of the hosts immune response to tumour, then subset manipulation as in the renal transplantation situation may be a therapeutic possibility.

However, much more work is required in dissection of the tumour host relationship before this becomes a practical proposition.

Wider Potential of Hybridoma Technology

While this article has concentrated on monoclonal antibodies to T lymphocyte subsets, hybridoma technology has much wider applications – many of potential interest to the urologist. An obvious area of interest is the identification and characterisation of tumour specific antigens. The initial excitement in this field however, has been tempered in the light of experience. Monoclonal antibodies raised against malignant melanoma [30] and breast carcinoma [25] have, when extensively screened for reaction with normal tissues or other malignant cell lines, been found to have weak cross reactivity. The controversy surrounding tumour specific antigens thus continues. The related hopes for tumour specific targeting of radiolabels or toxic agents must await monoclonals with greater tissue specificity. A recent report demonstrating that antibodies which bind to human glioma tumour cells are produced by lymphocytes within the tumour [26] offers some hope in this direction.

Monoclonal antibodies raised against blood group antigens may be of value in the investigation of the malignant cell membrane. This will be of particular relevance to transitional cell carcinoma of the bladder where tumour behaviour

has been correlated with loss of cell surface antigenic determinants [24].

Monoclonal antibodies have been produced, and are in the process of production, against a wide variety of hormones and enzymes important in the diagnosis of every day clinical problems. These reagents in combination with refinements of radioimmunoassay will dramatically improve specificity and shorten the time necessary for these assays. Finally they are also being used in the monitoring and purification of molecules of potential therapeutic importance such as interferon.

Conclusion

This article has outlined the technology involved in monoclonal antibody production. The examples of the application of this technology underline the outstanding importance of the original work done by Kohler and Milstein, who have surely earned themselves a prominent place in the history of science.

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