# Progress in Surgery

## VOLUME THREE

EDITED BY

I. Taylor

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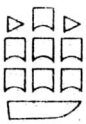

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# Preface

As surgical practice becomes increasingly specialized it is difficult for an individual to keep abreast of new developments in every field. The situation is especially problematical for the surgical trainee who is expected to be familiar with all innovations. The established consultant may not feel inclined to delve into fields outside his immediate specialized interest; however, every so often he may be required to express an opinion or provide advice and hence up-to-date information becomes necessary. 'Progress in Surgery' attempts to meet these needs.

In this volume aspects of both surgical science and practice are reviewed by experts with particular emphasis attached to true 'progress'. Some of the topics reviewed represent major surgical concepts important to all surgeons irrespective of speciality, such as modern immunology (P. Guillou) and wound healing (D. Leaper and M. Foster). Major progress has been made, and continues to be apparent, in the management of a number of traditionally troublesome disorders, for example, venous thrombosis (K. Burnand), adrenal disease (J. Farndon), parathyroid disease (R. Shields and S. Holt), anorectal problems in children (N. Freeman) and morbid obesity (R. Harrison and C. Clarke). Surgeons are understandably intrigued by technical advances which have a potential impact on patient management. Such interest has surrounded the surgical application of lasers (J. Carruth) and venous access (G. Sutton).

Finally, it is important to review those common disorders which are responsible for significant levels of morbidity and mortality. Improvements in management continue to occur in the following areas: oesophageal cancer (T. Hennessy), local recurrence of colorectal cancer (H. Umpleby), abdominal trauma (B. Rowlands) and intracerebral haemorrhage (D. Mendelow).

I would like to thank most sincerely all the contributors for giving of their time to write the reviews and the staff of Churchill Livingstone for their cooperation. Once again, I am most grateful to June Daniels for expert secretarial assistance and to Berry for her continuing patience and understanding.

Southampton, 1989 I. T.

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# Contents

# Modern immunology for the surgeon

In recent years there has been an explosion of knowledge concerning the physiology and pathophysiology of the immune system which will have profound effects on the practice of medicine within the next decade. These advances will influence surgical practice no less than other clinical disciplines and it is important that surgeons possess an appreciation of at least the basis of these developments. It is impossible to provide a complete review of a subject which owes its recent progress as much to geneticists and molecular biologists as to classical immunological investigators. It would seem appropriate therefore to give an outline of the current state of understanding of the various components of the immune system and their functional inter-relationships, before describing some advances in immunobiology which are of relevance to surgeons.

## THE PHYSIOLOGY OF IMMUNE RESPONSES

Immunological responses may be broadly categorized into those which occur naturally, without prior exposure to antigenic challenge, and those which are termed adaptive because they are specific to the invasive antigenic moiety which initiated the reaction. A summary of this division is provided in Table 1.1 but it can be seen that both types of host defence involve both cellular and humoral mechanisms.

### Natural immunity

Most immune responses involve a degree of acute inflammation, which takes place at the level of the capillary and pericapillary tissues. The physiology of acute inflammation has been well described and involves the participation of a number of locally acting factors which increase vascular permeability and activate the complement, kinin and coagulation cascades. Components of complement permit the adherence of particulate antigens or bacteria to phagocytic cells of the polymorph or monocyte series. Eventually specific antibodies also arrive to act as opsonins for such antigens but it should be noted that though antibodies may be critical for survival from

**Table 1.1** Classification of the immune response

| Type of response | Participating component | Target or function |
|---|---|---|
| A. Natural immunity | 1. Acute inflammatory response | Bacteria, trauma etc. |
| | 2. Opsonins, e.g. fibronectin | Bacteria<br>Particulate antigens<br>Parasites |
| | 3. Complement | As above plus<br>transplanted cells in association with specific antibodies |
| | 4. Phagocytic cells<br>fixed reticuloendothelial system | As above |
| | mobile monocytes and macrophages | As above |
| | 5. Natural killer cells | Tumour cells |
| | 6. Interferons | Viruses and tumour cells |
| B. Adaptive immunity | | |
| | 1. T-lymphocytes | |
| | (a) $T_D$ (delayed hypersensitivity) | Bacterial, parasitic and fungal antigens |
| | (b) $T_C$ (cytotoxic T-lymphocytes) | Transplanted cells<br>Tumour cells<br>Modified 'self' cells (e.g. viruses) |
| | (c) $T_H$ (helper T-lymphocytes) | Provide helper factors for other T- and B-lymphocytes to develop specific immunity |
| | (d) $T_S$ (suppressor T-lymphocytes) | Provide suppressor factors to dampen T- and B-lymphocyte responses |
| | 2. B-lymphocytes and antibodies | Production of antibodies against any 'foreign' antigen |

invasive organisms, they simply amplify and concentrate the pre-existing natural mechanisms.

Opsonization refers to the coating of a particulate antigen (e.g. bacterium) with a substance (opsonin) which facilitates the adherence of the particle to a phagocytic cell. Thus antibodies may act as opsonins by causing attachment of the particle/antibody complex to the $F_c$ receptor on a phagocytic cell (see below). Antibodies belong to the adaptive mechanism of the immune reactions but natural opsonins exist, including the C3b component of complement and an alpha$_2$ glycoprotein known as fibronectin. The complement system itself consists of a number of serum components whose sequential activation results in three distinct effects, namely adherence to phagocytic cells (C3 components), acute inflammation (C5 and C3 components) and, in association with specific antibody, lysis of invading cells whether bacteria or transfused or transplanted cells (C5b, C6, C7, C8, C9). In addition C3 and C5a are polymorph chemoattractants at sites of acute inflammation.

Phagocytosis is an important aspect of both natural and adaptive immunity. This function is served by a widely distributed system of vascular endothelial and reticulum cells together with various types of circulating and tissue-fixed macrophages. Although principally involved in the elimination of cellular debris and bacteria, it is now recognized that these cells serve the vital function of 'presenting' antigen to T- and B-lymphocytes so that adaptive mechanisms may proceed (see below). The cells capable of serving this function of antigen presentation are widely distributed and include microglial cells in the brain, Langerhans' cells in the skin and the so-called dendritic cells in the kidney and other organs, as well as the reticular cells in spleen, lymph nodes, thymus and bone marrow.

The physiological roles of interferon molecules are only just beginning to be elucidated. Three classes of interferons are so far identified. In addition to their well-known antiviral activities they possess other important attributes, some of which are summarized in Table 1.2. It will be noted that all three classes of interferon will activate natural killer (NK) cells but that only gamma-interferon will activate macrophages to become cytotoxic towards tumour cells. This is important because both these types of cell are active in preventing abnormal cells from expanding to become a distinct tumour and may possibly prevent metastatic tumour cells from establishing themselves in sites of election for metastasis. As will be described presently, their activity is impaired by trauma, including surgery.

**Table 1.2** The interferons

| | Alpha-interferon (Type I/leukocyte) | Beta-interferon (Type I/fibroblast) | Gamma-interferon (Type II/immune) |
|---|---|---|---|
| Source | Leukocytes | Fibroblasts only | T-lymphocytes only |
| Subtypes | 30 | 2 | 1 only |
| Mol. wt. | 20K | 26K | 17K |
| Stability | Acid stable | Acid stable | Acid labile |
| Inducers | Viruses Polynucleic acids (e.g. Poly I-C) | Viruses | Antigens<br>Mitogens<br>Interleukin-1<br>+ antigen<br>+ Class II 'self' |
| Properties | | | |
| Anti-viral | +++ | +++ | + |
| Growth inhibition | + | + | +++ |
| B-cell differentiation | | ++ | |
| Effects on Class I and II MHC-antigen expression | + | + | ++ |
| Activators of NK cells | ++ | ++ | ++ |
| Activators of macrophages | – | – | ++ |

## Adaptive immunity

Complex as the natural immune system may appear, recent developments in the investigation of adaptive immunity have revealed a system of cellular and molecular interactions whose integration is equally, if not more, intricate. Before discussing this system in detail it may be beneficial to recall several basic principles.

1. Antigens are molecules which are considered foreign by genetically different members of the same species or by a different species. 'Self' antigens are not normally recognized as foreign by an individual's immune system but they do act as part of the recognition system whereby mammalian organisms distinguish 'self' from 'non-self'. Thus 'self' antigens are only called antigens because they can be identified using reagents derived from animals of another species or immunogenetically different members of the same species who have been immunized against the molecule referred to as a 'self' antigen.
2. The functional sub-units of adaptive immunity are the 'T' and 'B' lymphocytes together with antigen-presenting cells of the monocyte/monophage series. The generation of an effective immune response depends on close cooperation between these cell types, this being achieved by the local release of soluble mediators and direct transmission of information in the form of a processed antigen. In general these soluble mediators are released and act only locally and thus may be termed paracrine immunological hormones.
3. Adaptive immunity is entirely dependent on the binding of molecules to specific receptors for those molecules and with no others. Such receptors may be present on cell surfaces (e.g. the receptor for the $F_c$ end of antibody, T-cell receptors for foreign antigen) or may be a component of another molecule (e.g. the $F_{ab}$, antigen-binding end of an antibody molecule). The fundamental point is that receptors are entirely specific for a single molecule (ligand).
4. B- or T-lymphocytes capable of mounting a specific immune response to a particular antigen do not normally exist in a definitive form within the immune system but are present as precursors (Fig. 1.1). These antigen-specific precursors may represent only an infinitesimally small proportion of the total lymphocyte pool whilst in the resting state, but following invasion of the animal with a specific antigen a cascade of events is initiated which results in the proliferation of that clone of T- or B-lymphocyte precursors which possess specific receptors for that antigen. These ultimately go on to generate vast numbers of effector cells such as cytotoxic T-lymphocytes or B-lymphocytes which mature into plasma cells manufacturing antibody with specificity for the invading antigen.
5. The recognition elements implicated in the immune response are genetically determined and the genes which play the most important roles in

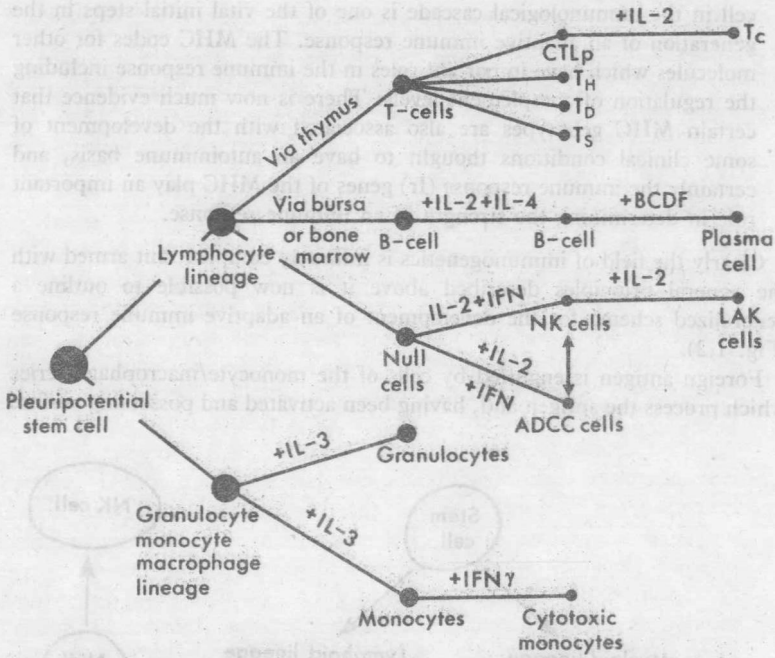


**Fig. 1.1** Schematic representation of the maturation of immunologically reactive cells from their precursors and bone marrow stem cells. IL-1, 2, 3, 4: interleukins 1, 2, 3 and 4; IFN: gamma-interferon; BCDF: B-cell differentiation factor; CTLp: cytotoxic T-cell precursor; Tc: cytotoxic T-cell; $T_D$: delayed hypersensitivity T-cell; $T_s$: suppressor T-cell; $T_H$: helper T-cell; NK cell: natural killer cell; LAK cell: lymphokine-activated killer cell; ADCC cell: cell mediating antibody-dependent cellular cytotoxicity

this sequence are those of the human major histocompatibility complex (MHC), present on the short arm of the sixth chromosome. A detailed description of the MHC is beyond the scope of this work but essentially the molecules coded for by this complex are recognized as the HLA (human lymphocytic antigen) system. These are the 'self' antigens described above and although originally described on leukocytes they have a ubiquitous distribution (though to different degrees) in all tissues of the body. Class I HLA antigens are present on most tissues and their importance lies in the fact that they are the molecules which T-lymphocytes recognize as 'altered self' when accompanied by processed foreign antigen. Class II antigens (also known as Ia or immune-associated antigens) are also vital recognition structures but are usually implicated in the initiation of the immune response at the level of antigen presentation. Hence quiescent macrophages do not normally express class II antigens but following activation with antigen are caused to do so. The presentation of processed antigen with 'self' class II antigen to the next

cell in the immunological cascade is one of the vital initial steps in the generation of an adaptive immune response. The MHC codes for other molecules which have important roles in the immune response including the regulation of complement levels. There is now much evidence that certain MHC genotypes are also associated with the development of some clinical conditions thought to have an autoimmune basis, and certainly the immune response (Ir) genes of the MHC play an important role in determining the strength of an immune response.

Clearly the field of immunogenetics is infinitely complex, but armed with the general principles described above it is now possible to outline a generalized schema for the development of an adaptive immune response (Fig. 1.2).

Foreign antigen is engulfed by cells of the monocyte/macrophage series which process the antigen and, having been activated and possessing surface

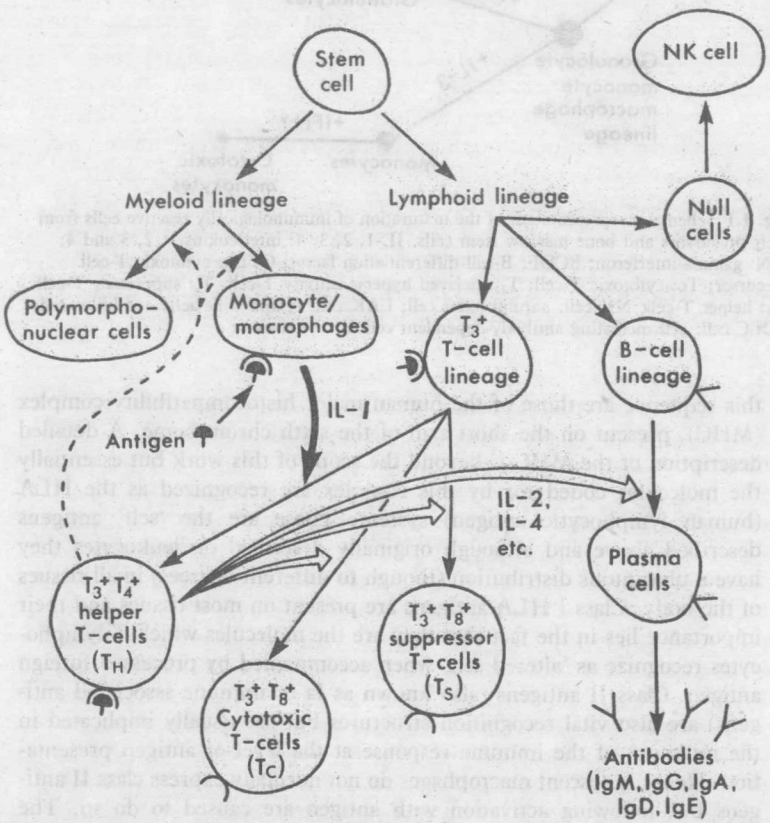


Fig. 1.2 Development of adaptive immune responses

molecules of the class II type (Ia molecules), these cells are able to present the antigen in the context of 'self' class II antigens to helper T-lymphocytes ($T_H$ cells). The 'self' class II antigens enable the recognition of the antigen presenting cell and, since $T_H$ cells possess surface receptors for the antigen in question, these cells also become activated. Simultaneously activated macrophages also secrete a cytokine known as interleukin-1 (IL-1) which binds to specific IL-1 receptors generated on the surface of activated $T_H$ cells. Concurrent binding of processed antigen to its specific receptors and IL-1 to its receptors on activated $T_H$ cells causes them to secrete a vital lymphokine, interleukin-2 (IL-2). The production of IL-2 is central to the generation of any immune response whether it be of the cellular type, mediated by T-lymphocytes, or the humoral type with the consequent maturation of B-lymphocytes which secrete specific antibodies against the eliciting antigen. Whichever response is generated, either T- or B-lymphocyte precursors bearing specific receptors for the antigen in question are presented with that processed antigen, by monocytes, macrophages or dendritic cells. These activated precursors are stimulated to express cell surface receptors for IL-2 and perhaps other $T_H$-derived helper factors of both antigen-specific and non-specific types. In fact $T_H$ cells generate a number of products which are essential co-factors for T- and B-cell maturation and differentiation into their mature effector cells. These molecules are classed as interleukins and play important physiological roles in maintaining adaptive immunity. Thus antigen-primed, cytotoxic T-cell (CTL) precursors, with high affinity IL-2 receptors, proliferate rapidly on exposure to IL-2 to produce an expanded clone of many thousands of cytotoxic T-lymphocytes, capable of killing any cell bearing the priming antigen. For example, in the case of viral infections such as influenza, CTL, which recognize the viral genomic products together with class I 'self' antigen, are produced in vast numbers, a process taking about five to seven days. Primed B-cell precursors which also express antigen receptors and a number of B-cell growth factor receptors (including receptors for IL-2) such as B-cell growth factor (interleukin-4), and B-cell differentiation factor, also undergo clonal expansion to develop into a large number of plasma cells producing antibody which is specific to the initiating antigen. The fundamental point here is that an adaptive immune response, whether it be of the cellular- or antibody-mediated type (often both co-exist), is a consequence of a cascade of cellular and molecular interactions which conspire to produce a large number of mature effector lymphocytes from a very small number of antigen-specific precursor lymphocytes by a process of rapid proliferation and maturation.

Over the years fundamental immunologists have been greatly pre-occupied with the mysteries which surround the basis of these responses in an effort to place them in a clinicopathological context thence to find means of manipulating the response. Two questions have been partly solved, namely the cellular origins of the cells implicated in the immune response

and the nature of the antigen receptors on T- and B-lymphocytes. It is now well-recognized that the leukocyte pool is replenished from the maturation of pleuripotential stem cells in the bone marrow which are capable of differentiating, under different influences, into cells of lymphoid (lymphocyte) lineage and those of myeloid lineage (Polymorphonuclear and monocytic cells) (Fig. 1.1). Those cells destined to become lymphocytes are further programmed in other lymphoid organs. Lymphocytes processed by the thymus (T-cells) are programmed to become those cells involved in the classical cell-mediated reactions such as delayed hypersensitivity responses ($T_D$-cells) or the 'first set' allograft reaction ($T_C$-or cytotoxic T-lymphocytes). It is noteworthy that up to 90% of the lymphocytes in the thymus gland are dead and perhaps these are the cells which alone recognize 'self' antigens (the forbidden clones) which are normally eliminated in the thymus. Cells which are not processed by the thymus are processed through an alternative organ which in birds is recognized as the bursa of Fabricius. Although the mammalian analogue of the bursa has not been identified with certainty, it may be the gut-associated lymphoid tissue or the bone marrow. Bursa-processed lymphocytes (B-cells) are those cells which will ultimately mature into antibody-producing B-cells and plasma cells. A third category of lymphocytes appears to possess neither T or B characteristics and are referred to as null cells. This lymphocyte sub-population constitutes the minority of peripheral blood lymphocytes but includes the very important natural killer cell system which has a physiological role in lymphopoiesis and regulating tumour cell growth. These cells can be identified and their function measured in vitro. They do not require specific pre-sensitization in order to kill their tumour targets.

The nature of the receptors for antigen on T-cells and B-cells differs greatly and long escaped elucidation. The T-cell receptor for antigen is a complex structure (the $T_3t_i$ complex) consisting of two major components which are inserted into the plasma membrane. On B-cells the antigen receptor appears to be homologous with the antigen binding ($F_{ab}$) end of antibody molecules (IgG or IgM) and explains why B-cells possess surface immunoglobulin.

Finally, as with all systems within the mammalian organism, regulating systems exist which dampen the adaptive immune response once its effects have been accomplished and are no longer required. For most types of immune reactions this suppressor effect is accomplished by T suppressor ($T_S$) cells via non-specific or specific humoral mechanisms. However, monocytes can also exert suppressor influences especially on NK cell system. In addition, Jerne's network theory of immunoregulation has been widely discussed and represents a system of auto-antibodies against our own antigen receptors (Auto-anti-idiotypic antibodies) which regulate antibody production by B-cells.

It should be appreciated that the above description is an abbreviated and greatly simplified overview of the immunological system. The complexity

of the system renders it liable to severe perturbation at any one of a number of steps in the cascade. These perturbations may be exploited, as in the case of organ transplantation, or represent a potentially lethal component of a patient's clinical condition, and I propose to review some of these scenarios which are of importance to the surgeon. Before proceeding to these, however, immunology research has provided two technical developments which are of such profound significance in all branches of medicine and biology that they deserve special mention. These are the development of monoclonal antibodies and recombinant DNA technology for the provision of large quantities of human molecules for scientific and clinical application.

## MONOCLONAL ANTIBODIES

When an animal is immunized with a complex antigen such as a human cell or even a single human molecule, e.g. human interferon, the animal generates antibodies against the antigen and acquires memory B-cells which will recognize the antigen and produce a more rapid and accentuated antibody response on re-exposure to it. These antibodies may be detected in the serum of the immunized animal. Unfortunately, such antigens are highly complex structures whose molecules possess not just one, but many sites (epitopes) which are antigenic. Thus the serum contains a mixture of antibodies, each one derived from a single B-cell clone and the antisera thereby obtained are described as polyclonal, consisting of a mixture of antibodies, each one of which may be present in small amounts and possess weak affinity for a particular epitope.

Antisera also contain antibodies against irrelevant components and are thus highly impure and are of limited value. Attempts to produce more specific antisera by refining the antigen tend to result in rather weak antibodies which are present in small quantities in xenogenic sera. How much better it would be if it were possible to 'capture' a single clone with its capacity for producing antibody of a single type with a single specificity, i.e. a monoclonal antibody.

This is precisely the technique that Kohler anl Milstein developed in 1975. B-cells do not grow very well in tissue culture and usually die off within a few days without releasing much antibody into the culture medium. In contrast, myeloma cells (malignant plasma cells) grow and proliferate very well in tissue culture and produce a monoclonal immunoglobulin which we recognize clinically as Bence-Jones protein. Clearly, if a single antigen-primed B-cell could be fused with the immunoglobulin factory of a myeloma cell, the resultant 'hybridoma' would respond to the DNA/RNA signals of the antigen-primed B-cell and produce vast quantities of monoclonal antibody against the priming immunogen. Furthermore, the immortality of the ever-dividing malignant hybridoma would ensure a continuous supply of cells which manufacture the antibody.

This technique is summarized in Figure 1.3. Spleen cells from an

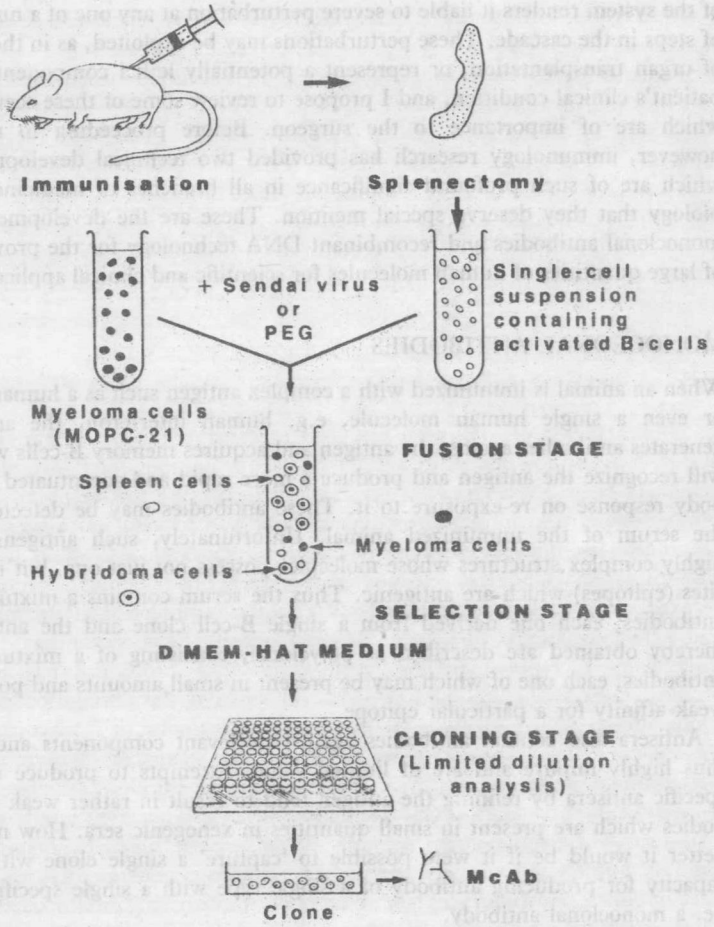


Fig. 1.3 Production of murine monoclonal antibodies

immunized mouse are incubated with a suspension of myeloma cells in the presence of a fusing agent such as Sendai virus or polyethylene glycol. A single B-cell fuses with one myeloma cell and the resulting cell suspension contains these hybridoma products together with unfused B-cells and myeloma cells. Using selective media, the unfused cells die off; there remains a suspension of hybridoma cells each one of which is capable of proliferating independently even if placed in culture on its own ('cloning out'). Single hybridoma cells are cloned separately and after a few weeks it is possible to detect which clone is producing the specific antibody (monoclonal antibody) of interest. When the clone is sufficiently large it produces vast quantities of the monoclonal antibody. The hybridoma may