ANAESTHESIA AND IMMUNOCOMPETENCE

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Despite continued advances in surgical and anaesthetic skills, infection in the period following surgery still remains a formidable problem. While a wide variety of factors is important in the pathogenesis of surgical infection—such as age, sex, race, nutrition, duration, urgency and time of operation, the collection of secretions and fluids, immobilization in bed, drug therapy, respiratory embarrassment, metabolic and electrolyte disturbances—in the ultimate analysis it is a depression of the body defence mechanisms which allows the invading infection to become established. The magnitude of the problem is revealed in a report of the Public Health Laboratory Service (1960), which suggested that in England and Wales the total excess stay in hospital as a result of sepsis approximated to 1 million days per year, or about 3% of the total bed-occupancy of acute hospitals. It is important that we acquire a better understanding of the normal defence mechanisms and the ways whereby they may be compromised by surgery or anaesthesia, or both. It may then be possible to manipulate the immune systems to gain beneficial effects during infection, metastasis, and transplant surgery.

THE IMMUNE RESPONSE

Memory, specificity and the recognition of the "non-self" form the core of immunology. It is beyond the scope of this review to cover this complex system, and an attempt will be made only to outline the most basic principles.

Cellular basis of the immune response

In all vertebrate systems, the small lymphocyte is the "basic unit" of the immune response, since it specifically recognizes the foreign antigen (Gowans, Gesner and McGregor, 1961). In response to infection in mice, antibodies are produced which can passively protect a second animal. Here, we have the establishment and transfer of humoral immunity. If, however, a mouse is given a transplant of foreign skin, serum from a mouse previously sensitized to the graft does not affect the rate of rejection. It is only after the transfer of lymphocytes from the sensitized mice that the rate of rejection resembles that of a "second set reaction". Hence, in graft rejection, antibodies are only secondarily involved, and the immune response is cell-mediated.

Two populations of lymphocytes

An anatomical and functional dissociation of the immune system into thymus-dependent or T-lymphocytes, and bursa-dependent or B-lymphocytes, was first proposed by Roitt and colleagues (1969).

T-cell system. This is composed of cells which are processed by, or in some way are dependent upon, the thymus, and is responsible for cell-mediated immunity. In the absence of exogenous antigen, cells migrate from bone marrow, enter the thymus and undergo rapid proliferation. Bach (1972) proposed that a "thymic hormone" influences the differentiation of bone marrow stem cells into T-lymphocytes within the thymus. Immunocompetence is achieved by the time the lymphocyte has entered the thymic cortex and is on its way to peripheral lymphoid organs. On appropriate stimulation by antigen, T-lymphocytes transform into lymphoblasts. These cells are concerned with the synthesis of their own components, but do not secrete appreciable amounts of free antibody.

B-cell system. B-lymphocytes are concerned in the synthesis of circulating antibody. The equivalent of the avian bursa of Fabricius has not yet been clearly defined in man and other mammals, although gut-associated lymphoid tissue such as the tonsil, Peyer's patches, lymphoid follicles themselves and haemopoietic tissue have been considered as possible candidates. The B-lymphocytes develop into the plasma cell series. Cell division and differentiation occur, again in the absence of exogenous antigen (Kincade and Cooper, 1971), while the mature plasma cells actively synthesize and secrete antibody. Figure 1 shows the lymphocyte differentiation pathways.

Cellular co-operation in the immune response

The role of macrophages. The cells of the monocyte-macrophage series play a central role in the induction of immune responses.
of the immune response. They trap and concentrate antigen at their cell surfaces for effective presentation to the lymphocyte, thus providing an accessory "second signal" in addition to the primary antigenic stimulus. Antigen taken up by free macrophages is partially degraded, but also fixed at or near their cell surface, where it is thought to be in a strongly immunogenic state. Concentration of antigen alone is possibly not the only mechanism by which stimulation is achieved. Some workers have suggested that a macrophage product (? RNA), unrelated to the specificity of the antigen, is providing an accessory signal for lymphocyte activation.

Co-operation between T and B cells

It is well known that the antibody response to certain antigens is considerably depressed following neonatal thymectomy. However, it is also recognized that T-lymphocytes themselves do not secrete antibody. This involvement of the lymphocyte in antibody synthesis, without itself producing antibody, is now seen to be the result of a form of co-operation by T-cells which helps the antigenic stimulation of B-lymphocytes to be more effective. Claman showed that both T- and B-cells were required in the mouse for a normal response to sheep red blood cells (Claman, Chaperon and Triplett, 1966). Further, Lischner and DiGeorge (1969) hold that no antibody is truly thymus-independent. This need for T-cell co-operation in B-cell antibody production has been extensively investigated by Mitchison (1971) in the hapten carrier situation. The antibody response of B-cells to hapten depends upon the existence of primed T-cells reacting with the carrier. Mechanisms postulated to explain such co-operation include:

(i) Presentation of the hapten in a multivalent form to cross-link B-cell receptors.

(ii) A second signal, independent of antigen, which could be mediated through a T-lymphokine (from T-lymphocytes) such as mitogenic factor, or by a complex through the Fc and C3 receptors.

(iii) Some degree of amplification, either by T-cell proliferation or by recruitment of macrophages by attachment of cytophilic antigen-specific receptors secreted by T-lymphocytes. Playfair (1971) has reviewed this complex subject at length.

Types of immune response

When antigen enters the body, two different types of immunological reaction may occur:

(1) The synthesis and release of free antibody into the blood and other body fluids. The antibody may act by direct combination with and neutralization of antigen such as bacterial toxin. Bactericidal antibodies together with complement, a complex system of enzymes activated during antigen-antibody reactions, lead to lysis of bacteria. Opsonins and agglutinins are important in preparing bacteria and fungi for phagocytosis. The mechanisms by which antibodies may afford protection in the external body fluids—tears, saliva, nasal secretions and those bathing the surfaces of intestine and lungs—are not yet fully elucidated.

(2) Cell-mediated immunity. This results in the production of "sensitized" lymphocytes which have receptors for antigen on their surfaces. However, the evidence that these receptors are immunoglobulin-like is poor, and their exact nature has not been established. These are the effector cells of cell-mediated immunity whose function is expressed in such reactions as transplant rejection and delayed hypersensitivity.
Non-specific immunity

Resistance to infection from an array of microorganisms is also dependent upon "non-specific" factors which can act independently of the immune system. For example, most bacteria fail to survive for long on the skin because of the direct inhibitory effects of lactic acid and fatty acids in sweat and sebaceous secretions, and the low pH which they generate. Ciliary actions in the respiratory tract prevent the inhaled dust particles and associated micro-organisms from reaching the alveoli. Mucus secretions, by competing with cell surface receptors for viral neuraminidase, can inhibit penetration of cells by viruses. The bactericidal enzyme, lysozyme, is abundantly present in the secretions of tears and saliva, and in granules of polymorphs and macrophages.

During the acute inflammatory response to foreign organisms, capillary permeability is increased, leading to egress of polymorphs and monocytes from the blood stream to the site where they combat the microbes by phagocytosis. There is also a massive release of serum bactericidal factors including C-reactive proteins, unrelated to immunoglobulins, which precipitate the group-specific C-carbohydrates of pneumococci in the presence of Ca^{2+}, and the complement system which may kill a variety of micro-organisms in the presence of Mg^{2+}. Complement may be activated by antigen-antibody in the "classical pathway", or by a variety of complexed protein in the alternative or "properdine pathway". The non-specific antiviral agent, Interferon, inhibits intra-cellular viral replication, having been itself synthesized by cells in response to viral infection.

Thus, we find that "non-specific" defence mechanisms are of vital importance, although the development of specific immune responsiveness remains indispensable for survival in normal individuals.

The interactions between non-specific (natural) and specific (adaptive) immunity are illustrated in figure 2.

Anaesthesia and the immune response

In most immune systems a wide variety of reactions may be affected by anaesthesia and surgery, this possibility being appreciated as early as 1875:

"... let us remember that chloroform does not act solely on the nerve tissue. Far from that, it has actions on all the tissues and attacks each one at a time which is a function of its susceptibility... An anaesthetic is not a special poison for the nervous system. It anaesthetizes all cells, benumbing all the tissues, and stopping their irritability ..." (Bernard, 1875).

Anaesthesia and the cell

The "cell", the "basic unit of life", obviously has a pivotal role in body defences and is susceptible to anaesthetic actions.

Biologists in the early part of this century reported that the cytoplasm of sea-urchin eggs becomes less viscous after exposure to ether, chloroform, paraldehyde, chloral hydrate and urethane (Heilbrunn, 1920). Seifriz (1941) observed that chloroform and cyclopropane reversibly stopped cytoplasmic streaming in the slime-mould Physarum polycephalum, finding this to be caused by a rapid and reversible gelation of the cytoplasm. It was suggested that the effect is the result of rapid and reversible locking of the linear protein molecules.

Goldacre (1952) and Bruce and Christiansen (1965) found that anaesthetic agents stopped movement of amoebae, which no longer responded to stimuli. These changes have been attributed to tight cross-linking of the colloidal constituents of plasma gel.

That anaesthetic agents can interact with proteins was revealed by Schoenborn, Watson and Kendrew (1965), and Schoenborn (1968), when they
demonstrated Van-der-Waal's bonding between xenon and cyclopropane and the proteins myoglobin and haemoglobin. Seeman and Roth (1972) observed that general anaesthetic agents in clinically used concentrations cause a 0.49% expansion of the areas of erythrocyte membranes. It has also been shown that local anaesthetic agents such as procaine decreased, while inhalation anaesthetics increased, the permeability of alkali metal cations in a lipid bilayer membrane system (Bangham, Standish and Miller, 1965; Johnson and Bangham, 1969; Johnson, Miller and Bangham, 1973). In view of these interactions with proteins, it seems possible that anaesthetic agents may affect the antigen and antibody reaction by altering the configuration of various molecular receptor sites.

Anaesthesia and cell motility

Inhalation anaesthetic agents decrease motility in a wide range of cell species. Nunn, Dixon and Moore (1968) observed a reversible dose-dependent effect on the locomotion of Tetrahymena pyriformis. A reversible inhibition of lymphocyte motility, on exposure to halothane, has been described (Nunn, Sharp and Kimball, 1970). Using time-lapse, phase-contrast cinemicrography, they were able to demonstrate a significant decline in lymphocyte velocity to a mean value of 0.6 μm/min, during exposure to halothane 2% for 1 hr. Since lymphocytes play a major role in the immune mechanisms, any inhibition of their function, however reversible, is likely to create favourable conditions for infective processes. Of further interest are the observations that mobilization of phagocytes is also adversely affected by anaesthesia. Bruce (1966) observed that halothane anaesthesia caused a decreased mobilization of neutrophils in response to intraperitoneal injection of pseudomonas endotoxin. In unpublished studies of phagocytosis, S. G. Kimball and J. Brody (1963) observed that focal accumulation of neutrophils in rabbit ear skin windows, was markedly depressed after anaesthetizing the animals with ether or halothane. Lowenburg (1934) also had observed similar depression of locomotion in vitro. Although these studies suggest that anaesthesia depresses macrophage migration, the evidence is not entirely conclusive. Nunn, Sharp and Kimball (1970), during their lymphocyte motility studies, observed that macrophage locomotion did not seem to be affected by halothane.

To combat an invading organism, the macrophages must move and have a sense of direction. At present, little is known about the effects of anaesthesia on chemotaxis.

Anaesthesia, phagocytosis and intracellular killing

Phagocytosis is a primary defence mechanism against infection. Abnormalities of neutrophil function remain the most important variable of immunological defence against infections (Alexander, 1972). Since there is evidence that anaesthetic agents render cells immobile, it is conceivable that they may affect phagocytic activity also. Indeed, as early as 1911, Graham showed an inhibition of phagocytosis when human and rabbit leucocytes were exposed to ether. Hamburger, in 1916, reported a dose-related inhibition of phagocytosis by equine leucocytes in vitro after exposure to chloroform. Recently, Bruce (1967) showed that halothane anaesthesia caused a substantial reduction in the number of salmonella bacteria ingested by each peritoneal neutrophil, 4 hr after i.p. injection in mice. Kosciolek (1967) reported a decrease in phagocytosis in blood obtained from surgical patients after halothane and ether anaesthesia. Leucocytes from these patients immediately after surgery, and 24 hr later, exhibited a decreased ability to phagocytose Staphylococcus aureus.

Cullen, Hume and Chretien (1972) reported a decrease in phagocytosis of latex particles and nitroblue tetrazolium (NBT) reduction in patients during either halothane or nitrous oxide–narcotic anaesthesia without surgery. In a later study (Cullen, 1974), however, halothane 0.5–2.5% or nitrous oxide 80% produced only minimal, statistically insignificant, inhibition of latex particle phagocytosis or NBT reduction. Their data corroborated earlier in vitro work by Rosenbaum and Orkin (1973), who failed to detect an inhibition of yeast particle phagocytosis by human neutrophils after exposure to halothane. Cullen (1974) has suggested that the inhibition of phagocytosis reported in vivo during anaesthesia might result from other factors, such as stress or altered blood flow. It is worth recalling that in vivo professional phagocytes preferentially ingest particles coated by opsonins. Recent studies have demonstrated that IgG receptor sites are present on the cell surfaces of monocytes and neutrophils, but the latter cells require complement in addition to IgG in order to accomplish efficient phagocytosis (Douglas, 1970). It might be speculated that anaesthetic agents hinder opsonization or alter the cell receptor sites.

Anaesthesia and cell division

Östergren (1944) showed that most anaesthetic agents caused a dispersion of metaphase, which was indistinguishable from the effects of colchicine. Nunn, Lovis and Kimball (1971) observed a similar develop-
ment of C-mitosis in botanical species under the influence of anaesthesia.

Lassen and colleagues (1956) observed clinically that, following long-term therapy with nitrous oxide for treatment of tetanus, patients developed depression of cell growth in the bone marrow. Green and Eastwood (1963) reported similar marrow depression in rats given nitrous oxide for a prolonged period. Bruce and Koepke (1966) observed that, in contrast to the nitrous oxide effect on bone marrow, halothane did not diminish overall cellularity. In a later study of halothane (Bruce, Koepke and Taurig, 1968) it was found that the ratio of dividing to maturing cells was increased, suggesting a damming effect on the dividing cells of the myeloid series. Bruce and Taurig (1969) provided further evidence that there is inhibition of DNA synthesis with a delay in the S-phase.

Fink and Kenny (1968) reported dose–response curves for inhibition of growth of mouse heteroploid cells and mouse sarcoma I cells by various anaesthetic agents. Jackson (1972) has observed also a dose–response relationship for inhibition of growth of mouse hepatoma cells by halothane. Most recently, Sturrock and Nunn (1975) have investigated effects of various inhalation anaesthetic agents on the division of Chinese hamster fibroblasts, observing a dose-dependent inhibition of cell multiplication with all agents tested. Halothane increased the cell cycle time roughly in accordance with its effect on multiplication rate, and also caused a marked and rapid reduction in the prophase count, suggesting prolongation of the G2 (post-synthetic phase). Also, frequent delay in the division of cytoplasm at mitosis was observed.

Thus, it seems that anaesthetic agents act on every phase of the cell cycle. Since proliferation of sensitized cells of lymphoid tissue, upon contact with the appropriate antigen, is the key event in the immune response, anaesthesia may result in depression of the immunocompetence by suppressing the reactions whereby cells divide. It seems possible that anaesthesia affects both specific and non-specific components of immunity, by modifying the functions of the cell.

**Anaesthesia and infection**

The possibility that anaesthetic agents may alter the course of an infection has been under consideration for the past 70 years. In 1903, Snel observed increased mortality in guineapigs infected with anthrax, after exposure to ether, chloroform and chloral hydrate. Rubin (1904) made similar observations in rabbits infected with streptococci or pneumococci and exposed to ether or chloroform. Both the depth and duration of anaesthesia were important in increasing the severity of these infections. In 1910, Opie anaesthetized dogs with chloroform, with or without concomitant bacterial infection, and studied their livers. He observed that he had "...succeeded in producing lesions of a character and intensity not obtained by simple administration of chloroform." While these early studies seem to suggest that anaesthesia may enhance an infection, other workers have observed a reverse effect. Waterhouse (1915), a surgeon from Charing Cross Hospital, suggested a beneficial effect of diethyl ether in cases of pyogenic infection. Also, a bactericidal effect from ether vapour was noted by Topley (1915).

Bronfenbrenner and Weiss (1924) reported that anaesthetic agents, alone and in combination with specific antitoxins, decreased mortality from experimental botulism. Kaspar (1928) found a higher survival rate (37%) in intoxicated animals given antitoxin and tribromoethanol, compared with only 4% survival in animals which received antitoxin alone. Sulkin and colleagues (Sulkin and Zarafonetis, 1946; Sulkin, Zarafonetis and Groth, 1947) studied the influence of ether on experimental neurotropic virus infections, and showed that it afforded protection to weanling mice infected with the Eastern equine encephalomyelitis, Western equine encephalomyelitis and St Louis encephalomyelitis group of viruses. More recently, Bruce (1967), in the study of effects of halothane on salmonella peritonitis in mice, observed that the time of death in anaesthetized animals was significantly sooner than in the unanaesthetized animals. Goldstein and colleagues (1971) found that cyclopropane and methoxyflurane reduce murine pulmonary bactericidal activity.

In addition to a possibly deleterious effect of anaesthetic agents on defences against bacterial infection, there remains the topic of viral infections. In an initial study of the influence of halothane on mortality from murine heptatitis virus (MHV3), Moudgil (1973) reported a statistically significant increase in mortality in the anaesthetized animals, and a 10-fold reduction in the LD50 dose compared with the control groups. Subsequently, the same author examined the influence of halothane on mortality at various time intervals before and after infection with 1, 10 and 100 LD50 doses of murine hepatitis virus (Moudgil, 1976, in preparation). The studies demonstrated a significant increase in the mortality of the anaesthetized groups when anaesthesia...
was given immediately before or up to 24 hr after infection; however, no significant difference could be observed when the animals were anaesthetized 48 hr after or up to 24 hr before infection. Thus, we observe that anaesthesia alters the course of infection and increases morbidity and mortality. The complex mechanisms which come into play require closer and further examination.

**Anaesthesia and the humoral antibody**

There is virtually no information on the effects of anaesthesia on antibody production in man. Using the Jerne plaque technique (Jerne, Nordin and Henry, 1963), it was observed that 24 hr of anaesthesia with halothane, pentobarbitone or nitrous oxide reduced the concentration of antibodies in the sera of rats (Wingard, Lang and Humphrey, 1967). In this species, recovery of normal antibody-forming capacity did not occur up to 72 hr following exposure to anaesthesia (Humphrey, Wingard and Lang, 1969a). The same authors compared the effect of halothane with that of surgery (Humphrey, Wingard and Lang, 1969b). Both surgery and anaesthesia were associated with a reduction in antibody-producing splenic cells. This suppression lasted for 48 hr after exposure to anaesthesia and was not detectable at 72 hr. The mechanism of these effects has not been clearly established and may be related to stress or hypothermia. Clearly, more work is desirable in this field.

**Anaesthesia and lymphocyte immunocompetence**

The ability of peripheral blood lymphocytes to transform and divide under stimulation by a mitogen, for example phytohaemagglutinin (PHA), is a measure of their immunocompetence (Bloom, 1971). This is usually measured by their ability to incorporate tritiated thymidine into DNA, during lymphoblast transformation. Alternatively, their ability to kill the target cells against which they have been immunized forms the basis of cytotoxicity reactions, thus providing an indication of immunocompetence.

Evidence for depression of immunological competence following surgical procedures performed under general anaesthesia is now extensive. Studies have revealed a decrease, in the period immediately after surgery, in the responsiveness of peripheral blood leucocytes to PHA (Riddle and Berenbaum, 1967; Park et al., 1971), to tumour antigens (Cochran et al., 1972; Vose and Moudgil, 1975) and to bacterial antigens (PPD) (Berenbaum, Fluck and Hurst, 1973). These clinical studies do not permit a separation of the effects of duration and depth of anaesthesia, surgical trauma and length of operation, each of which correlated with the depression of transformation. Some authors have reported that surgical procedures were necessary to evoke a reduction of immunological reactivity in the guineapig following barbiturate anaesthesia (Cooper, Irvine and Turnbull, 1974), while others have observed a decrease in PHA responsiveness in children following induction of anaesthesia but before surgery (Espanol, Todd and Soothill, 1973). *In vitro* equilibration of lymphocytes produced a reduction in PHA responsiveness, but the prolonged presence of halothane was required in order to achieve this (Cullen, Sample and Chretien, 1972). Bruce (1972) also observed similar dose-dependent inhibition of PHA responsiveness with halothane. On the other hand, in an unpublished study no effect on PHA response was observed in blood withdrawn from volunteers at the end of a prolonged anaesthetic without surgery (B. F. Cullen, 1974; personal communication).

Although the implications of depression of immunocompetence are obvious, future studies of this system must attempt to clarify the respective roles of anaesthesia and surgery.

**Anaesthesia and tumour immunology**

In the present state of knowledge, any discussion of immunology of human tumours can be concerned only with potential application rather than practical reality. In studies of cell-mediated immunity to tumour cells in man, the existence of tumour-associated antigens has been suggested by the results of several groups of workers (Currie, Lejeune and Fairley, 1971; Hellström et al., 1971). From these studies, it appears that the neoplastic cell is antigenic and that the cell-mediated immune responses of the host tend to inhibit tumour growth. Therefore, patients with immune deficiency states show an increased incidence of malignant disease (Fialkow, 1967). Iatrogenic immune deficiency is also associated with the development of tumours. Patients undergoing intensive immunosuppression, especially with anti-lymphocyte serum, tend to develop lymphoreticular malignancies (Penn et al., 1969). Studies of the effects of anaesthesia alone on "tumour takes" in animals are contradictory. While Agostino and Clifton (1964) observed that anaesthesia increased pulmonary metastases in rats, Schatten and Kramer (1958) reported that there was no significant effect of anaesthesia, operation or cortisone on the number of pulmonary metastases following i.v. injection of S-91 melanoma cells in mice. As noted above, surgical
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procedures under anaesthesia result in immunosuppression in the early postoperative period. Although direct evidence implicating anaesthetic agents is not available, it remains a distinct possibility that these may play a major role in tumour metastasis. Further work with animal models should provide information in this relatively unexplored field.

CONCLUSION

The evidence presented in this review leaves little doubt that anaesthetic agents may influence a wide variety of specific and non-specific host defences. Clearly, we must acquire a better understanding of the mechanisms whereby these tools of our trade are likely to produce deleterious effects in terms of morbidity and mortality.

Patients presenting for surgery may already be receiving drugs which may result in immunosuppression, as is the case with steroids (Scotthorne, 1956; Nicol and Bilbey, 1958; Thompson and van Furth, 1970), with cancer chemotherapy (Gabrielson and Good, 1967), with hydantoin (Grob and Herold, 1972), with anti-complementary agents, for example aminocaproic acid (Rowinski and Hager, 1966), and with antibiotics (Tarnawski and Batko, 1973). These are only a few of a large number of pharmacological agents which may be encountered. Other factors such as age, nutritional deprivation (Cannon et al., 1945), fever (Ellington and Clark, 1944) and viral infections (Notkins, Mergenhagen and Howard, 1970) also have an important effect on immunosuppression. An appreciation of the exact role played by each factor is of paramount importance.

By understanding the nature of these deficits in body defences, it may be possible to prevent infection and morbidity, when methods for manipulating the immune response become available.

REFERENCES


