CHAPTER I

REVIEW OF LITERATURE
A. THE IMMUNOLOGICAL INTERACTION IN NEOPLASIA

One of the most frequently quoted concepts from Burnet's theory of "Immunological Surveillance" (1965) is that development of malignant tumor must represent failure of the host's defense mechanism. In other words, the escape of the tumor from the surveillance may be a consequence of some form of immunological failure on the part of the host. This has been amply confirmed from increased incidences of the tumor in immuno-deficient patients either naturally occurring or iatrogenic. Thus it became evident from long term investigations that an immunosuppressed condition or an immuno-deficient state strongly favours a neoplastic development in the host, and, also, conversely, a developing malignant tumor have got a profound immunosuppressive influence on the host (Currie, 1974). The early experiments as well as investigations conducted recently have furnished conclusively that the components of immune apparatus suffer a great deal of functional deficiency during neoplastic development in the host (Currie, 1974; Cochran, 1978; Woodruff, 1980).

Apart from a number of mechanisms operated by oncogenic viruses for malfunction of cellular immunity, large number of tumor materials are involved for immunosuppression which has been reported as phenotypic modifications of tumor cell antigens; classically these are antigenic modulations (Boyse and Old, 1969), shielding of antigens (Gold and Friedman, 1965; Thomson et al., 1973; Currie and Alexander, 1974), blocking factors (Moller, 1964; Hellstrom and Hellstrom, 1969; Basham and Currie, 1974) etc.
Since the later decades of the last century the possible role of immunological responses in resistance to the development and growth of malignant tumor has been subjected to extensive speculations and some experimentations. In fact, the concept of tumor resistance was crystallized by Ehrlich (1906) although he attributed it to nutritional factors. Presence of specific antigens on experimental tumor was even hinted at by several transplantation experiments such as those by Cloes and Baeslack (1905) which was later modified by Woglom (1929) with a notion that tumors possessed antigens capable of eliciting specific immunological responses in the host and that they can evolve both the humoral and cell mediated components of immune apparatus.

A complex net-work of cells and their products interact to perform a variety of diverse functions necessary for the generation, expression and regulation of many levels of specific and nonspecific immunity in course of tumor development. A possible effector mechanism in tumor immunity is now almost certain and have been described by many (Currie, 1974; Cochran, 1978; Mitchison and Landy, 1978; Woodruff, 1980; Weters, 1978-81; Swain, 1983). The principle components those are involved in tumor immunity can be categorised in the following way.

A. **Humoral** which includes (1) opsonisation and phagocytosis (2) complement mediated lysis (3) loss of cell adhesion and,

B. **Cellular Immunity** including (1) direct lysis by cytolytic T-cells, (2) Antibody dependent killer or K-cells and antibody dependent cell mediated cytotoxicity (ADCC), (3) activation of macrophages by factors released by T-cells, and non-specifically activated macrophages, (4) Natural killer cells (NK) and (5) Lymphokine activated killer cells (LAK), very recently role of polymorphonuclear neutrophils (PMNs) in tumor immunity have also been discussed (Katano and Torisu, 1982; Sendo et al., 1986).
A. Humoral Responses

The first evidence for the existence of tumor specific antigens (TSA) was provided by Gross (1943), who showed that a fibrosarcoma induced by chemical carcinogen, methylcholanthrene (MCA), would not grow in syngeneic mice that had been immunised by small amount of tumor before challenge. Antibody directed towards these antigens have been demonstrated in serum of immune animals bearing variety of experimentally induced tumors. Currie and Sine (1973) showed that syngeneic immune serum will inhibit in vitro movement of lymphoma cells. That specific humoral factors may play a role in inhibiting development of metastasis has been indicated by Proctor, Rudenstam and Alexander (1973). Although a number of such antibodies (humoral factors) have now been useful in serologic characterisation and the isolation of tumor associated antigens (TAA), the presence of humoral factor is consistently correlated with increase tumor resistance in the host. Nevertheless, there are several ways in which tumor specific antibodies could theoretically mediate anti-tumor activities.

(1) Opsonisation and Phagocytosis

Opsonisation is the binding of specific antibody and complement component with particulate antigen to facilitate phagocytosis. In vitro studies have demonstrated the ability of macrophages to exert cytotoxic activity against some tumor cells by cytophagocytosis in the presence of immune serum which is now believed to contain a material, opsonin. The subject has been widely reviewed by Koren (1983). However, relevance of this activity in vivo is difficult to assess.

(2) Complement mediated lysis

There are reports that in human tumors complement dependent cytotoxic antibodies occur in the serum of tumor bearing patient with certain diseases. In malignant melanoma, Lewis and his colleagues (1969) have detected cytotoxic (lytic) antibodies in patient with localized disease which disappeared with the progression of the tumor. Currie (1973) has shown that following immunisation with irradiated tumor cells, patients
with disseminated malignant melanoma and hyper-nephroma develop circulating antibodies which, under appropriate in vitro circumstances, are capable of lysing these specific target cells. It has further been reported that tumor specific antibodies develop in early disease and following tumor excision and such antibodies can be made to lyse tumor cells under artificial conditions. The overall observation, however, suggests that tumor cells are rapidly destroyed in the presence of complement. Susceptibility of this type of lytic attack is dependent on the target cell types. Demonstration of tumor specific complement dependent cytolytic antibodies in tumor bearing subjects has become increasingly difficult when tried in in vitro experiments. In fact, when these antibodies are tested in culture, they are often reported to lack the particular cytotoxic activity. However, this highlights another problematic aspect associated with these antibodies' assay, for under rather special circumstances, such as prolonged incubation with sources of complements, these antibodies can be shown to have cytotoxic properties.

(3) Loss of cell adhesion

The ability of antibodies to attach to the surface of tumor cells in vivo is considered important in exhibiting antitumor activities other than those mediated by complement dependent lysis or phagocytosis by macrophages. It has been suggested that antibodies bound to the membrane of the malignant cells may modulate surface structures and thereby interfere with cell adhesive properties (Benjamini et al., 1982). Furthermore, adherence of circulating tumor cells to the endothelium of blood vessel appears to precede metastatic spread. Antibodies specifically bound to the tumor cells may result in loss of adhesive properties important to the establishment of blood-borne metastatic foci.

B. Cell Mediated Immune Responses

The alternative effector pathway of the host responses is via cell mediated or cell associated mechanisms in which the lethal confrontation between tumor cell and immune response is vested in the properties of specialised effector cells. Failure to passive transfer of tumor immunity with serum was first described in the early years of this century, and this
failure constituted the main distinction which could be drawn at the time between tumor immunity and immunity to the bacteria. Many histological studies have indicated to cellular infiltration seen around the tumors and it was not until the work of Murphy(1926) that showed lymphocyte was incremimated as a possible mediator of immunological reaction to grafted tumor cells.

From Biologists' point of view the engagement of immune response with antigenic tumor cells seems to be a highly evolved form of cellular interaction. These are now well known to be associated with the cell surface receptor concept and consequent recognition phenomena and, in fact, involve the presence of some macromolecular structures(Nicolson, 1976). Based on evidences from neutralisation and immune adaptive transfer experiments(Herberman, 1974), cell mediated immunity is thought to be the most important defense mechanism against neoplasia(Burnet, 1971). The various cell mediated immune mechanisms have been reviewed recently by Brondz(1972), Cerottini and Brunner(1974), Hellström and Hellström(1974) and Herberman(1974). Considering the details of the discussions made by them and also on the basis of some recent developments(Thomas et al., 1983; Unanue et al., 1984) there appears to be at least six basic mechanisms of cellular interactions against the tumor.

1. Direct lysis by cytotoxic T-cells (CTL)

This type of T-lymphocyte now characterised as T-8 lymphocyte as per monoclonal specification(Thomas et al., 1983), proliferate and differentiate into blast like cells during an immune response to tumor associated antigens (TAA). The recent studies on these cytotoxic T-cells revealed that prominent antigens which are capable of eliciting cytotoxic T-cell responses are cell surface products of the major histocompatibility gene complex(MHC), viruses, and tumor specific antigens.

Two basic models have been advanced(Henney, 1977) to account for the MHC restriction of the action of cytotoxic T-cells. One of these envisages a single T-cell receptor directed against MHC-encoded molecules that have been modified by their physical interaction with the 'foreign' antigens. This model has been designated as 'Altered self.' The second model proposes
(two T-cells receptors, one for foreign determinant and the other for the MHC gene product. However both the models share the concept that cytotoxic T-cells bear a receptor that can specifically occupy or engage the antigens. In fact the processes triggered by antigen occupation of T-cell receptor account for the lytic activity of cytotoxic T-cells.

The recent understanding of the exact mechanism of T-cell mediated cytotoxicity has been mainly derived from the study of K.T.Brunner and his collaborator(Cerrottini and Brunner, 1974). Classically the mechanism has been verified with $^{51}$Cr release assay when the sensitized lymphocytes were allowed to react with $^{51}$Cr labelled target cells for short term culture. Although this system involved the lysis of allogenic cells, mechanistically it is considered to be identical to the means by which cytotoxic T-cells destroy virally infected autochthonous tissue or syngeneic tumor cells. Thus the serial steps through which cytotoxic T-cells function have been generalised on the basis of several investigations put forth(Kolb and Ganger, 1968, 1970; Perlmann and Holm, 1969; Cerottini and Brunner, 1974; Allison, 1974; Furluga and Allison, 1974; Henney, 1977); three separate aspects of the "Lytic cycle" have been considered: (1) Cell - Cell interaction simply hits at the binding between the target antigen and the lymphocyte receptor. The adhesion of effector cells to their target has been shown to be inhibited by cytochalasin-B, which reveals that this effector target contact phenomenon is accompanied by membrane modulatory movement which further requires presence of magnesium ions(Mg$^{++}$); (2) Lesion insertion - Subsequent lytic phase of cytolysis is apparently energy independent. It has been shown that membrane permeability of the target cells changes within minutes following effector adhesion. It is not exactly known how long the cytotoxic T-cells "sit" over the target cell before moving on to cause further damage. These events are absolutely dependent on the presence of Ca$^{++}$. cAMP concentration has been found to show an inverse relation with extent of cytotoxic function by CTL. (3) Destruction of target cell membrane - Events of target cell lysis are terminated with the onset of membrane lysis of the target cell. This stage of membrane lysis has been classically demonstrated by several investigators(Cerrottini and Brunner, 1974; Henney, 1977).
As a result of collision with cytotoxic T-cells, the target cell undergoes a series of membrane permeability changes ending in rupture of its membrane. These have been confirmed using markers of varying molecular size and isotopes to tag the internal cytoplasmic materials as indicators of target cell destruction, e.g., $^{86}$Rb efflux, $^{51}$Cr release and $^3$H-thymidine DNA imigration from the target cells. Several findings as such, suggest that the initial lesion caused by CTL allows rapid exchange of inorganic ions and small molecules but not macromolecules. The latter being able to pass the membrane only after secondary effect on the cell resulting from disordered osmotic regulation. The eventual demise of the target cells appears to be caused by colloid osmotic forces resulting from water influx (Henney, 1977).

2. Antibody dependent cell mediated cytotoxicity: K-cells

When tumor target cells are exposed to specific antibody in tissue culture they become susceptible to lysis by lymphoid cells from an unimmunized donor (Moller, 1965; McLennan et al., 1970; Perlmann and Perlmann, 1970). This observation was originally made by using various preparations of lymphocyte suspensions as the effector cells and the antibody was consequently called 'lymphocyte dependent-antibody' (LDA). Studies continued thereafter showed that these were not T-cell population and rather a type of cytotoxic B-cell line (McLennan, 1972). The recent work, however, does not confirm the concept of being the B-cells as the particular cytotoxic type and at the moment they are being referred to as 'killer cells' or K-cells by some authors. Thus, the operation of a quite distinct cytotoxic mechanism is suggested by Perlmann's finding that target cells coated with low concentration of IgG antibody can be killed through an extra cellular nonphagocytic mechanism involving unsensitized lymphoreticular cells which bind to the targets by their specific receptors for the C2 and C3 domains of IgG Fc (Perlmann et al., 1972, Perlmann et al., 1974). This specialised type of cytotoxic reaction against the tumor has been referred to as antibody dependent cell mediated cytotoxicity (ADCC) and is now considered to be exhibited by both phagocytic and non-phagocytic myeloid cells (Polymorph and monocytes) and by a weakly glass adherent cell with Fc receptors, the so called K-cells (Greenberg et al., 1973; Greenberg et al.,...
Although these are considered to be identical with some cytotoxic lymphoid series, at least in certain features, they still maintain the refined specificity with the help of Fc receptor for the desired cytotoxic potential. However, the precise lineage of the K-cell is still uncertain. A population of human effector cells bear T-markers and therefore belong to the granular T-G subpopulation, the remainder are 'null' cells in the sense that they lack the presently employed surface markers of mature B- or T-lymphocytes and they still await a proper evaluation whether they belong to an entirely distinct cell type (Van Boxel et al., 1972; Trinchieri et al., 1975). As observed, these effector cells are abundant in the peripheral blood, spleen and peritoneal cavity of experimental animals and are deficient in thoracic duct lymph and lymphnodes. In man, they are readily detectable in the peripheral blood.

The LDA or the lymphocyte-dependent antibody, as mentioned earlier, being responsible for ADCC reactions are mainly of the IgG type in most systems so far described. The most notable feature of LDA phenomenon which is attractive to immunologists is its exquisite economy in the use of the immunoglobulin molecules. Perlmann et al. (1972) have indicated that as few as hundred IgG molecules are adequate to lyse a chicken red cell.

Another attractive feature of this phenomenon is the nonspecific requirement for effector cells. With the help of scanty specific IgG a potent lytic effect can occur in those sites where the effector cells are available. Basham and Currie (1974) have shown that in rats implanted with a chemically induced fibrosarcoma specific LDA lytic activity (ADCC) is readily detectable in the serum with the first two weeks of tumor growth, at a time when no complement dependent cytolytic activity can be detected. As the tumor progresses this antibody activity disappears and it is replaced in the serum by free tumor specific antigens. Contact between the effector and target cells is essential and activity is inhibited by cytochalasin-B which interferes with cell movement and aggregated IgG which bind firmly with Fc receptors and finally blocks the ability to interact with the antibody on the surface of the target. The reaction is not affected by inhibitors of protein synthesis nor does it require presence of complements.
So far, ADCC has been studied exclusively as a phenomenon in vitro, its role in vivo still remains a question.

3. Activation of macrophages by factors released by T-cells

It has been recognised for sometimes that macrophages are involved in the afferent limb of immune response. In recent years it has become almost certain that macrophages phagocytose the antigens and process it in some way that they are presented in a highly immunogenic form to the other cells of the lymphoid series (Unanue et al., 1984). However, it has become further important with the fact that cells of these monocytes – macrophage series may also constitute an important effector mechanism in both allograft rejection and tumor immunity (Fink, 1976; Koren, 1983). The notable role of macrophages in tumor immunity has been reported quite long ago and macrophage infiltration in tumor area with a favourable prognosis has been reported by Black et al, as back as 1954. Borer (1961) had suggested that macrophages were involved in the efferent limb of immune reaction to tumor, but his ideas were not followed up until much later. Evans and Alexander (1970) have shown that macrophages immunised with lymphoma cells are capable of specifically inhibiting the in vitro replication of the appropriate lymphoma cell lines. Furthermore, they have shown that specifically cytostatic and cytolytic macrophages can be produced by "arming" of normal macrophages by exposure to immune lymphoid cells. The immune lymphoid cells when cultured with the target cells release a supernatant factor which is capable of specifically arming the macrophages. It has further been reported that during encounter with the appropriate specific target cell the 'armed macrophages' transform in both morphology and function. These transformed macrophages are hyperactive vacuolated and appear angry. Furthermore, their cytotoxic properties become non-specific in that they cause cytostasis of a variety of unrelated tumor cells in vitro. Similar activated macrophages can be detected in animals infected with intracellular parasites such as Toxoplasma gondii (Hibbs, 1973). Surprisingly, it has been shown that cytostasis effect of activated macrophages thus obtained is immunologically non-specific but that it is only active against malignant cells; their benign counterpart remains unaffected. Similarly activated macrophages can be produced by treatment of normal peritoneal exudate macrophages (PEM) in vitro.
with low doses of endotoxin (Perr et al., 1973).

The induction of cytotoxic macrophages by tumor-immune T-lymphocyte has been studied in a series of experiments. It has been shown that lymphocyte obtained from mice immunised with irradiated lymphoma cells were not active themselves against the immunising tumor cells in vitro. However, macrophages obtained from these same immunized donors were specifically cytotoxic to the lymphoma target cells. Furthermore, peritoneal macrophages isolated from the normal syngeneic mice could be rendered cytotoxic following incubation with spleen cell from immunised donors. Evans and Alexander (1972) showed that lymphocytes immunised in vivo influence macrophages by their products, including migration inhibition factor (MIF), macrophage spreading factor (MSF) and specific macrophage activation factor (SMAF) and also a cytophilic T-derived material that makes macrophages more receptive to antigens (Feldman, 1969). The T-cells which are considered responsible for such activation of macrophages by their humoral products are most non-toxic in nature. Although SMAF mediated macrophage activation is known to tumor specific recognition and tumor killing, the specific mechanism is not clear. It has been suggested SMAF may be an immunoglobulin like T-cell product that specifically absorbed to macrophages and possesses a recognition site for the immunising tumor antigens, thereby imparting specificity to the ensuing cytotoxic events another way by which macrophages could specifically kill tumor cells. Once they become activated by immune T-lymphocytes the killing effect is mediated by antitumor antibodies (ADCC).

Hadden and Stewart (1981) showed that immune lymphocytes can elaborate over 50 regulatory activities collectively known as lymphokines and quite an important number of these are responsible for macrophage activation. Some of these lymphokines attach macrophage to the site of immunologic reaction, prevent their migration away (MIF), and stimulate them with MAF to undergo morphologic changes resulting in enhanced oncocytolytic capacity. As these type of enhanced killing capacity has been successfully demonstrated against variety of tumor target cells, the macrophage appears to be important non-specific effector of an antigen specific cell mediated
response, the factors that convert macrophages to the tumoricidal cells is distinct from SM4F and can be obtained from the supernatants of sensitized lymphocytes also containing MIF activity. The very recent activities have shown that most macrophage activating factor (MAF) activity present in lymphokine rich culture supernatant are due to interferon gamma (IFN - \( \gamma \) ) (Sanders and Littman, 1986). Further enhancement of macrophage mediated phagocytosis by cytokine incubation (lymphocyte) has also been hinted by Chakraborty et al. (1986). The recognition system mediating this form of tumor killing is unknown.

The oncocytolytic process mediated by macrophages appears to be the same whether they are from donors immunised specifically with tumor or with general immunostimulant. It has been demonstrated that the destruction of tumor cell, by macrophage requires intimate contact between the two cells. As far as this type of killing is concerned phagocytosis does not appear to be an important phenomenon, although they have got great role in tumor reduction by levelling secondary lysosomes with indigestible non-toxic agents. It was shown that activated macrophage directly transfer the contents of its lysosome into the target cells following membrane fusion between the two cells, the efficiency being dependent on the amount of cytocidal lysosomal enzymes delivered to the target cells. It has been reported by several investigators that soluble cyto-toxic or cytostatic mediators are present in the culture supernatants of activated macrophages, however, the nature of these factors and their mechanism of action is not well understood (Koren, 1983).

Several animal model system have been useful in determining the ability of macrophages to modulate tumor growth in vivo. Particularly animal injected with immunostimulants known to enhance in vitro macrophage cytotoxic activity have reduced incidence or delayed appearance of chemically induced tumor. Based on comparative studies of the contents of the macrophages of different tumors, it has been claimed that a direct relationship exists between the degree of macrophage infiltration and reduction of metastatic rates, although it has not been ruled out that they are simply involved in the 'Clean up' of cellular debris (Fidler and Poste, 1982).
4. Activated macrophages by different stimuli

When peritoneal exudate macrophages (PEM) are activated by various stimuli, they are found to show the ability to resist the tumor growth in vitro system. These types of macrophages are usually discussed in separate heading because these are supposed to be stimulated by various non-specific stimuli. Such activated macrophages are found in increased concentration during chronic infections, due to the induction to macrophages by exposure to lymphokine by addition of agents such as endotoxin or double stranded ENA (Alexander and Evans, 1971). The tumor cells are lysed for over a period of 48 hours by these activated macrophages as has been demonstrated by Currie and Basham (1975). The peculiar property of such activated cells are their tumor-discriminating action, leaving the normal cells unaffected. However, this action of tumor lysis has been found to be associated with the soluble supernatent factor with a low serum concentrations; with higher concentration of serum the lytic effects seems to require cell to cell contact. The mechanism of action of such activated macrophage against tumor are still being searched out and its mediation by various biological products for therapy of malignancy has been critically discussed (Currie, 1976; Mantovani, 1983; Ishida, 1986).

Dendritic Cells

Another category of cells recognized at present also appear to participate in some immune inductive events. The cells vary in their minute anatomical structure with respect to their origin. The Langerhans-dentric cells are found in abundance in the epidermal layers of the skin and also in the deep cortex of lymph node. They derive from stem cells of the bone marrow and migrate to the epidermis. The cells show low level of Fc and C3 receptors, can bind to antigens and have Ia antigens on their surface. They are also believed to move by way of the efferent lymphatic to the deep cortex of lymph nodes. Further, in spleen and lymph nodes, a dendritic cell akin to the Langerhans cell has been isolated and studied for its immunological activities. All these cells have been found to be active in presenting some antigens to T-cells and to be very effective stimulators of the mixed lymphocyte reactions (MLR). The exact features and immunological
functions have been discussed details by Steinman and Nussenzweig (1980).

Recently some dendritic cells from the intestinal-lymph nodes of tumor immunised mice have been found to show considerable immunity and antitumor activity against the specific targets (Gyure et al., 1987) the precise nature of these dendritic cells (Macrophage like) is being investigated.

Following antigenic stimulation a specialised population of cells have been found to be emerging from the regional lymph node draining the site of antigenic inoculation. In sheep, Hall and Morris (1963) detected them in efferent lymph node while in rat Deloreme et al. (1969) found them in thoracic duct lymph. In man these types of cell have also been detected in peripheral blood by Crowther et al. (1969). Morphologically they have been characterised as large lymphoid cells resembling lymphoblast and have been called immunoblast. They can even be detected in lymph and blood in response to a variety of tumor antigens including tumor specific transplantation antigens (TSTA). Even by adoptive transfer these sensitized cell lines has also been reported to confer a high degree of specific antibody production. They also exhibit the powerful cytotoxic effect. The recent years, studies in the laboratory of J.G.Hall have extended these findings with the involvement of some lymphatic dendritic cells as mentioned earlier (Gyure et al., 1987). Much emphasis is now being given in order to establish the tumor specific role of lymphatic immune effector cells in cancer bearing patients.

Natural killer cells

As established from the recent studies in tumor immunity, the major cell lines involved in defending the tumor development are principally of (a) lymphoid origin and (b) of monocytoid origin. The first category of lymphoid series involving the role of T-cells along with helper and the suppressor types and that of B-cells have large been discussed. A third category of cells which are now considered to be of lymphoid origin have been given much importance recently. These are now known as natural killer cells, named after their property of killing the tumor cells spontaneously.
even the absence of T-cells (Herberman et al., 1973, 1980). Although some studies argued about the designation of natural killer cells (Klein, 1983), it has been reported that these cells exhibit a natural cell mediated reactivity against tumor cells. Morphologically these cells have similarities with large granular lymphocyte and, further, both the cells have been found to play the role in killing the YAC-1 Lymphoma cells. It seems, therefore, that NK cells might have a similar morphology to that of LGL (Luyni et al., 1981).

The recent details of NK activities and their character have been reviewed by Woodruff (1986) and Uchida (1986). Much have been discussed regarding the kinetics of NK cells towards target lysis. A number of studies using Cr$^{51}$ release assay have shown that they have a natural cytotoxic effect on the target cells which means that they do not require any pre-sensitisation with the particular target in the host concerned (Stutman et al., 1978; Herberman and Holden, 1978; Burton, 1980; Woodruff and Hodson, 1985). These studies have shown that NK cells can be subdivided into two categories according to the differences of duration of target cell lysis event, namely the type-1, which can be detected in 4 hours assay; and the type-2, which are characterised in 18—29 hours assay. However, there might be overlap between two (Lattime et al., 1983). While studying the natural cytotoxic capacity of NK cells, it has been reported by many that these cells can function as K cells using the specific antibody raising against the specific target, that is, capable of performing ADCC reactions (Herberman, 1980; Woodruff and Hodson, 1985).

Several attempts have been made to identify cell surface markers on subsets of NK cells by different assays (Glimcher et al., 1977; Cantor et al., 1979; Burton et al., 1982). Accordingly, type-1 corresponds roughly to NK$_A$, NK$_I$ or simply NK cells and those of type-2, NK$_B$, NK$_S$ and NC cells. All these are reported to show various surface antigens including NK-1, Thy-1 (50%), Ly-1 (25%), Ga-3, Ga-4, Ga-5, Ly-5, Ly-6, Ly-10, Ly-11 and Asialo-GM$_1$, which are not expressed by the type-2 cells. Whereas Mac-1 may be expressed by both types. At least some NK cells appeared to be pre-thymic, which, in the presence of a thymus can differentiate into T-cells.
A number of evidences suggest that the NK cells belong to the T-cells lineage (Scheid et al., 1975; Herberman and Holden, 1978; Kindred, 1982; Acha-Orbea et al., 1983; Yanagi et al., 1985; Reynolds et al., 1985; Robertson, 1985).

The level of NK activity is influenced by a number of factors including age and genetic background of animals and also that of man (Herberman et al., 1973; Petranyi et al., 1976; Herberman and Holden, 1978; Clark et al., 1980). However, a variety of experimental manipulations are held responsible to influence NK activities in man and animals (Woodruff, 1986; Uchida, 1986). Type-1 activity is increased by interferon (IFN) and some IFN inducers including BCG (Herberman et al., 1977) and C-Parvum (Ojo et al., 1978). Further, some other components like Poly(I), Poly(C), Poly(A), Poly(U) have also been found to be inducers. Remarkably, tumor cells can induce IFN and can stimulate NK activity (Stutman et al., 1980). Type-2 activity has been increased by IL-3 (Djeu et al., 1982). Role of IFN has been given much importance for spontaneous activity in young mice. Thus NK sensitive tumors can be eliminated by a dose specific IFN treatment. Further, several other agents like phytohaemaglutinine (PHA), concanavaline A (Con-A), other lectins, few microbacteria etc (Woodruff, 1986) are found to stimulate NK activities under different conditions. Some important inhibitors have also been mentioned, including cyclophosphamide, hydrocortisone prostaglandin (PG-E₁, PG-E₂), silica, and C-Parvum when given intravenously. Irradiation, starvation, neurological lesion and development of tumors are also considered inhibitory to NK activities.

Recently the cytolytic and regulatory function of NK cells have been reviewed in detailed in human neoplasia, in which the role of NK's has been particularly highlighted in autologous tumor killing (ATK) (Uchida, 1986).

**Polymorphonuclear Neutrophil (PMN)**

The granulocytes of different categories have been believed to have their long association with different tumor systems. Cells of the polymorphonuclear series might have an important role in arming or activating cell co-operation with humoral mechanism (Dvorak et al., 1978; Korec et al., 1980; Gerrard et al., 1981). The well known association of polymorpho-
nuclear neutrophil (PMN) can be regarded as circumstantial evidence for the involvement of these cells in tumor immunity (Kikuchi et al., 1974). During the initial stage of tumor transplantation, they found an increased accumulation of PMN surrounding the tumor mass which, however, is gradually replaced by small lymphocytes. Although not clear the exact role of PMN in tumor immunity is still controversial and awaits further evaluations. However, several authors utilised the PMN status in terms of their phagocytic activities as the tumor conditions in the host (Rosner et al., 1970; Cline, 1973; Pickering et al., 1975). Recently the mechanism of the tumor cytotoxic effect exhibited by polymorphonuclear neutrophil has been explained by Sendo et al. (1986) on the basis of a lymphocyte factor activating the neutrophils (neutrophil activating factor, NAF).

**B. IMMUNOPOTENTIATION AND THERAPEUTIC APPROACH IN NEOPLASIA**

The urge for stimulating the immune system has long been appreciated by many under a variety of pathological conditions including cancer. As it became evident from numerous studies that immunity in cancer is a potent factor that controls the growth of the tumor in the host (Evans, 1986), the concept of maintaining the immunity under the conditions has been given considerable importance (Currie, 1974; Moller, 1982; Ishida, 1986). On the contrary suppression of immunity by any means has been found to augment the development of tumor in the host (Evans, 1986). Results of conventional therapy indicate repeatedly that new approaches are urgently required especially when radiation or chemotherapeutic measures or selective surgery towards malignancy become deleterious to the systemic immune status in the host. Although criticised by some (Cochran, 1978) it is widely accepted that immunopotentiation by several agents under the conditions can play an important role in controlling the tumor growth.

The attempts of immunopotentiation have been undertaken quite a long time ago, and, during the latter part of the 19th century and the early part of the 20th century (Colei, 1891-93; Nauts et al., 1946) it has been shown that reticuloendothelial stimulation by infection with some micro-organisms and micro-organisational products were capable of tumor regression.
Following these studies and many more of the recent studies the concept of immunotherapy has emerged into the management of cancer problem with the indication of the way of hopeful therapeutic measure. Currently many therapeutic approaches are under trial in the human patient with malignancy either applied alone or as combined along with radio- or chemotherapeutic agents.

At present, ideas about increasing the immunosensitivity of the tumor targets are nebulous. Various chemical and enzymatic materials are used for this purpose. Depending upon their interpretation of the experimental and clinical evidences, immunotherapy divide readily into two groups: (i) which includes reticular endothelial system by general stimulation and has been termed active nonspecific immunotherapy and (ii) which might require secondary immunisation against TAA to which a week reaction is already existing, or, raising of a new immune response to TAA which have not spontaneously induced the immune response; this has been termed as active specific immunotherapy. Two other forms of immunotherapy using sensitising agents like DNBC or DNFB to tumors, and, second, passive immunotherapy involving the humoral antibodies and specifically sensitised lymphocytes but there also remained considerable interests in the adoptive transfer of immune materials by administering substances such as immune RNA (Fink, 1976) or transfer factor from individual with regressed tumor or who have been immunised to a cancer.

Of the different methods employed for immunisation, active specific immunotherapy is probably the method of most use. The requirement here is a strong stimulant of the immunological apparatus which carries no major side effect(s) or morbidity and which on present evidence should probably preferentially stimulate cell mediated immunity (Currie, 1974; Cochran, 1978). The agents employed divide readily into biological agents, whole microorganisms, or materials derived from them. These agents are recently considered as biological response modifiers (BRM) (Talmadge, et al., 1985; 1986).
The most widely used material is Bacillus Calmette Guerin (BCG), which is an attenuated bovine tubercle bacillus and is the biological adjuvant and has received most attention therefore. Early studies indicated that an infection with virulent mycobacterium tuberculosis was associated with an augmentation of humoral immunity (Lewis and Loomis, 1924) and cell mediated immunity (Dienes, 1936). Further studies have shown that exposure to mycobacterial materials causes ranging effects in vivo. These include increased resistance to foreign organism, increased strength of allograft rejection and increased resistance to tumor challenge (Biossi et al., 1954; Old et al., 1961; Baldwin and Pimm, 1973). Some in vitro studies further exhibited increased phagocytosis (Biossi et al., 1954), stimulation of macrophages for killing the tumor targets (Hibbs, 1972; Evans and Alexander, 1972; Cleveland et al., 1974), overall increased cell mediated immunity (Littman et al., 1973; Mackaness et al., 1974) and increased circulating T (E-rossetting) cells (Serrou et al., 1975). An increased mitogen responsiveness by lymphocyte has been reported by some authors (Cohen et al., 1974) but has criticized by others (Gutterman et al., 1973; Golub et al., 1976). These various observations actually stimulated most studies in the elucidation of BCG mediated potentiation against cancer. A better stimulatory effect of BCG as a biological adjuvant has earlier been shown by Freund (1956) who introduced the fraction of heat killed mycobacterium tuberculosis in mineral oils for the purpose.

Reports on the use of BCG against human cancer followed, initially in acute lymphoblastic leukemia (ALL) (Mathe et al., 1963) and then in other leukemias and "haematosarcomas" (Mathe et al., 1967). The clinical application of BCG in man has been started in 1960 in case of different malignant problems and is continued still now. The British Medical Research Council Leukemia Committee (1971), however, compared the maintenance activity in BCG and methotrexate treated patients with acute lymphoblastic leukaemia and found BCG inferior to methotrexate as far as treatment measure is concerned. Mathe, as mentioned earlier, however, observed a positive finding towards use of BCG under the condition. The difference in these findings between MRC and Mathe has been explained on the basis of the
difference in the mode of trials conducted in terms of source, dosages, methods and route of administration adopted for the purpose.

As far as mechanism(s) concerned, BCG have been found to stimulate different cell lines involved in immunity against cancer. Former studies indicated that the reticuloendothelial system is by far the mostly stimulated component in tumor immunity (Currie, 1976). Wide range of use of BCG particles for general immunopotentiation has, however, some contraindications (Nathanson, 1972; Pinsky et al., 1972; Haunt et al., 1973; Serrou et al., 1975). Although they are capable of reducing the tumor burden in a large number of malignant patients (Mathé, 1976), the random use of BCG has been limited due to the occurrence of pathological complications. These include formation of abscesses followed by production of pus over a period of weeks or months at the site or near the vicinity of the injecting position; moreover, liver biopsy examination of BCG recipient showing hepatic dysfunction has exhibited tuberculous granuloma in the liver granulomas hepatitis and various other skin lesions including melanomas in a considerable proportion of patients. Thus care must be exercised due to this incompatibility of BCG introduction with unscheduled doses under which circumstances the mycobacteria may spread from their site of introduction to the draining lymphonodes. Even death has also been recorded as a result of anaphylactic reactions to BCG, associated with high fever and major coagulation failure (McKhann et al., 1975).

The second most important agent that has been described to have a better effect in tumor regression is corynebacterium parvum now renamed as Propionibacterium acnes (C. Parvum, P. acnes) (Milas and Scott, 1978). The interest in the bacteria as an immunostimulant was first derived after the demonstration of Halpern and his co-workers (1969) who showed that the killed organisms into mice caused intense stimulation of the lymphoreticuloendothelial system. The stimulating effect to immunological network with this material has been comparable with that of BCG and some related components which were supposed to stimulate the reticuloendothelial system only and not much of the lymphoid partner (Scott, 1974a). C. Parvum (CP) has been found to have remarkable effect in experimental animal including
the activation of macrophages to augmented phagocytosis, chemotaxis, and enhance killing of tumor targets (Halpern, 1975). There is less argument on the effect of CP on lymphocytes but some reports describe the stimulation of B- and T-lymphocytes and increase in antibody synthesis against T-dependent and T-independent antigens and also enhanced CTL activity (O'Neil et al., 1973; Howard et al., 1973; Zola, 1975). Evidence of activation of cellular immunity has been described by Scott (1974b, 76) in terms of assessing the DTH reaction. Other reports of depression of cell mediated immune response to unrelated antigens have also been received (Allwood and Asherson, 1972; Scott, 1974a; Castro, 1974a; Colapinto, 1975).

C. Parvum has been found to show antitumor activity against a wide range of animal tumors (Halpern et al., 1966; Woodruff and Hoak, 1966; Lamensans, et al., 1968; Currie and Bagshawe, 1970; Smith and Scott, 1972; Israel and Halpern, 1972; Woodruff and Dunbar, 1973). The observed effects were, however, dependent on the size and type of tumor, the route and time of administration of CP.

Many trials of CP have been made in human malignant disease and at least in some cases successful reports have been made. The initial studies have been made as combined therapy along with chemotherapeutic analogs (Israel and Halpern, 1972; Israel, 1973; 1975; Israel and Edelstein, 1975). Other reports of combination therapy following irradiation (Mathe et al., 1975), following surgery (Ishmael, et al., 1976) have also shown some increased survival and tolerance of chemotherapy and radiotherapy under the conditions.

The toxicity of CP has also been reported in various occasions. These include short febrile reactions, pain at the site and near vicinity, local inflammations and suppuration, hepatotoxicity and many more (Israel, 1975; Woodruff et al., 1975; Reed et al., 1975). Mild cardiovascular changes with transient hypo- and hyper tension, diffuse intravascular coagulation, thrombocytopenia and nephrotic syndrome may also develop in certain percentages of CP recipients (Reed et al., 1975).
An antihelminthic drug, Levamisole was investigated in animals and found to have the effects on functions of lymphocytes and macrophages, restoring their normal level of action in case of depressed immune status. Thus the immunostimulating capacity of the agent has been verified in tumor bearing animals—with somewhat conflicting results. A majority of authors found no antitumor activity (Fiedler and Spitler, 1975; Hooper et al., 1975; Johnson et al., 1975) but some have reported inhibition of tumor growth (Sadowski and Rapp, 1975; Takarura, 1975); few others observed enhanced tumor growth (Fiedler and Spitler, 1975; Mantovani and Spreafico, 1975). Inspite of disappointing records in animal models, attempts have been made, on the basis of its recorded availability, to enhance humoral and cellular immunity with consequent antitumor activities (Shibata et al., 1975; Holmes and Golub, 1976). Although few former records are negative (Ward, 1976), a number of recent observations are mostly in favor of levamisole as anticancer agents (Rojas et al., 1976; Advani et al., 1979; Spreafico, 1980). However, they have shown few contra-indications.

A few more materials have shown antitumor activities in terms of immunopotentiations. Of these some mitogens, antilympholytic serum (ALS), B Group vitamins, DMCB, Poly-IC etc. have shown more or less antitumor action in different experimental models (Cochram, 1978).

The recent discoveries of immunopotentiators and their use in reducing the tumor burden have further inspired immunotherapists for continued study of biological materials as anticancer agents. The 'Biological response modifier' or 'Biomodulators' as they are called by recent nomenclature have already occupied the field of immunotherapy with a much greater satisfaction (Talmadge et al., 1985; 1986). Of these, interleukin-1 and (IL-I, IL-II) (Marz, 1987), interferons (Taylor-Papadimitriou, 1985) are being used regularly in both experimental and clinical trials with recurrent successes. OK-432 or picibanil, a material derived of streptococcus pyogenes have recently shown a very potent immunostimulating capacity with subsequent antitumor effects (Ishida, 1986). The recent line of studies indicated that NK cells and LAK like cells are activated
Some former reports have also been made concerning adoptive immunotherapy by transfer factor (Lawrence, 1955-1969; Rapaport and Lawrence, 1975; Levin et al., 1975). Immune RNA (I-RNA) are some specialised RNA molecules of 7S-11S dimensions and are reported to exhibit tumor regression when isolated properly from specifically sensitised host cells (Fishman, 1961; Pilch et al., 1975; Fink, 1976; Steele et al., 1981).

It, therefore, brings the general consideration that a number of biological materials are dominating the field of immunotherapy of cancer and a considerable number of these are particulate in nature. Apart from BCG, C-Parvum and others corpuscular material like Sheep erythrocytes (SRBC) have recently been reported to exhibit antitumor activity (Chaudhuri et al., 1986). However, the exact mechanism and its mode of action still remaining unveiled and requires extensive studies for further confirmation of its role as an antitumor immunostimulant in both experimental and clinical subjects.