LECTURE: 13

Title: TUMOUR IMMUNOLOGY

LEARNING OBJECTIVES:

The student should be able to:

- **Explain the term "immune surveillance".**
- **Identify weather surveillance acts against viruses or tumours.**
- **Briefly explain the cellular responses to tumour-associated antigens.**
- **Discuss the mechanism of detection of tumour-associated antigens by immune cells.**
- **Discuss the mechanism of detection of tumour associate antigens by antibodies.**
- **Explain the human tumour immune responses and escape mechanisms.**
- **Describe the tumour as a tissue graft.**
- **Identify the role performed by the monoclonal antibodies in detecting tumour antigens expressed on tumour cells.**
- **Briefly explain the passive immunotherapy with monoclonal antibody.**
- **Briefly explain the active immunization as immunotherapy.**
- **Briefly discuss the role of tumour markers in the diagnosis and treatment of malignancy.**
- **Discuss the tumour immunodiagnosis (Tumour markers).**

LECTURE REFRENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE IMMUNOLOGY. 6th edition. Chapter 18. pp. 289-301.
2. HANDOUT.

Tumour immunology

- Immune surveillance is a concept that envisages prevention of the development of the most tumors through early destruction of abnormal cells by the host's immune system.
- Surveillance probably acts against viruses not tumors. The evidence for this is that although there is an increased incidence of tumors in immunosuppressed individuals, the most dramatic increase is in tumors associated with oncogenic viruses.
- Cellular responses to tumor-associated antigens occur; the antigens may be virus coded, or they may be altered or over-expressed host gene produce.
- Differentiation antigens expressed on tumor can be detected by monoclonal antibodies or patients' sera. Although the antigens are no restricted to tumor cells, they are useful for diagnosis and my be targets for antibody-mediated therapy.
- Passive immunotherapy with monoclonal antibodies is promising when single cells are targeted or the problem of poor penetration into tumor masses can be circumvented.
- Immunotherapy by active immunization or by passive transfer of cells is still largely experimental, because of tumor escape mechanisms. Cytokines are active against a few tumor types.

THE TUMOUR AS A TISSUE GRAFT

The idea that there might be immune responses to tumors is an old one. At the turn of the century, Paul Ehrlich suggested that in humans there was a high frequency of 'aberrant germs' (tumors), which if not kept in check by the immune system would overwhelm us. Thus tumors came to be regarded as similar to grafted tissue recognizable by the immune system. This, in turn, led to attempts to stimulate the immune system to reject them. Occasional regressions following treatment with bacterial vaccines (Coley's toxin), or occurring spontaneously, were taken as evidence of an effective immune response.

Early in the century, experimentalists began to investigate tumor immunity and noted that transplanted tumors usually regressed. Although much of this work fell into disrepute when it was realized that the immune response was directed against foreign major histocompatibility complex (MHC) antigens, it did establish that the immune system can reject a large tumor mass when there is an antigenic disparity between tumor and host. Only in the post Second World War period, when genetically homogenous inbred rodents became available, did it become possible to investigate the immune response of animals bearing a tumor expressing identical MHC antigens. An added impetus to these studies was provided by Burnett and Thomas, who developed Ehrlich's idea of immune responses to 'aberrant germs', elaborating it into the theory of immune surveillance.

IMMUNE SURVEILLANCE

Surveillance is the most effective against viruses not tumor cells

Burnett and Thomas' idea was that the immune system continually surveyed the body for the presence of abnormal cells, which were destroyed when recognized. The immune response to a tumor was therefore thought to be an early event, leading to the destruction of the majority of tumors before they became clinically apparent. It was also proposed that the immune system played an important role in delaying. A variety of evidence was adduced to support these ideas:

- Postmortem data suggest that there may be more tumors than become clinically apparent.
- Many tumours contain lymphoid infiltrates and in some tumors this may be a favourable sign.
- Spontaneous regression of tumors occurs.
- Tumors occur more frequently in the neonatal period and in old age, when the immune system functions less effectively.
- Tumours arise frequently in immunosuppressed individuals.

Although at first sight this appears impressive evidence in favour of the theory, on closer examination the strongest point, the association between immunosuppression and increased tumour incidence, is less conclusive. The largest body of data comes from the study of kidney transplant recipients, many of whom have been followed for over 20 years. The frequency of many tumor types is increased in this population (**Figure-1**), and in several of these there is strong evidence that a virus may be involved (**Figure-2**). There is also a slight but definite increased risk for many other cancers in which viruses are not known to play a role. This suggests that the immune response may be most important in preventing the spread of potentially oncogenic viruses and that surveillance against other non-viral tumors is relatively ineffective. Certainly, normal humans who become infected with EBV carry the virus for life and make strong cellular and humoral responses to the virus. Increased virus replication and shedding of viral particles in secretions, occurs in immunodeficient individuals, so that it is clear that the immune response limits virus replication under normal circumstances (**Figure-3**).

Animal experimental data support the view that immune surveillance is largely directed towards viruses rather than tumors. Studies of athymic nude mice or mice immunosuppressed with anti-lymphocyte serum, did not show a general increase in tumor frequency. However, a high proportion of the mice developed tumors cause by viruses (introduced in the anti-lymphocyte serum), which seldom cause tumors in normal animals. When tumors were induced by the carcinogen methyl cholanthrene in SCID (Severe Combined Immunodeficiency) and normal mice, there was no difference in tumor incidence, but the tumors arising in SCID mice were more immunogenic. These data suggest that a functional immune system prevents oncogenesis by viruses but not the development of non-viral tumors. Once a tumor has arisen, less immunogenic variant cell may be selected by an anti-tumor immune response.

How important are microorganisms for human cancer?

In man relatively few tumor types are known to be caused by viruses (Figure-4), but liver and cervical cancer cause over a million deaths worldwide, implying that hepatitis and papillomaviruses must be able to evade immune surveillance in normal individuals. A recent addition to the list of microorganisms associated with tumors is the bacterium *Helicobacter pylori* in stomach cancer. Increasingly, stomach cancer does not show a great increase in immunosuppressed individuals. Unlike oncogenic viruses, *H. pylori* is not an intracellular pathogen and cannot directly transform cells so that the relationship between the pathogen, epithelial cells that are potential targets for transformation, the host response and other genetic and dietary factors is likely to be complex.

TUMOUR ANTIGENS

Tumour antigens may be detected by immune cells or antibodies

Viral antigens may be targets for cellular and humoral immune responses but thee is also abundant evidence of genetic alternations (mutation, gene amplification, chromosomal deletion or translocation) in most, if not all, tumors. Some of these lead to the expression of altered molecules in tumor cells and other to over-expression of normal molucules. These changes may be demonstrated either by detecting the host immune response or experimentally by deliberately immunizing other species with tumor.

Tumor-associated antigens detected by immune cells

Tumor antigens were first demonstrated by transplantation tests. When a tumor was grafted onto an animal previously immunized with inactivated cells of the same tumor, resistance to graft was seen. Tumor resistance, subsequently shown to the mediated by immune cells, was directed at tumor-associated transplantation antigens (TATAs) of two types. The first are antigens that are shared by many tumors (T antigens), even though these may not even be of the same tissue of origin. The second are antigens that are specific to an individual tumor (tumor-specific transplantation antigens - TSTAs). Tumors may express both specific and shared antigens.

Shared Tumor antigens are of viral origin

These antigens are found on tumors induced by viruses such as the small DNA polyoma and SV40 viruses, which can cause tumors in experimental animals, and the papillomaviruses, which are implicated in human cervical canver. These viruses code for T (tumor) antigens that are shard by other viruses of the same group. T antigens are nuclear proteins, which play a role in the maintenance of the transformed (cancerous) state. Herpes viruses cause tumors in many species including man in the case of EBV.

In animals, infectious RNA oncogenic viruses cause leukaemias and sarcomas, and at least one human leukaemia virus (HTLV 1) has been discovered (**Figure-4**). These viruses bud from the cell membrane of infected cells, and the viral envelope's glycoprotein can be detected at the host cell membrane. There are strong humoral and cell-mediated responses to antigens of DNA- and RNA-tumor viruses (**Figure-3**), which can protect against tumor challenge. Because tumors produced by a given oncogenic virus all share the same antigen, mice immunized, say, with SV40 virus-induced tumor cell reject a different SV40-induced tumor but are susceptible to one induced by polyoma virus.

In some strains of mice, activation of endogenous RNA tumor viruses occurs regularly, leading to leukaemia. In others, when carcinogenic chemicals are given, the resulting tumors may express vital antigens and produce infectious mouse leukaemia virus (MuLV). Such tumors express common tumor-associated antigens as well as the tumor-specific transplantation antigens (TSTAs) discussed below. Host immune responses to endogenous RNA viruses are weak, perhaps because of immunological tolerance.

Tumor-specific transplantation antigens are due to alternations in tumor genes or gene expression

TSTAs are antigens which can induce tumor rejection, but the animal must have been previously immunized with the same tumor (**Figure-5**). These antigens were first detected by chemical carcinogens and their nature has now been elucidated, as described below:

A transplantation (therefore poorly immunogenic) tumor of inbred DBA2 strain mice was exposed *in vitro* to a powerful mutagen. This produced mutant subclones, some of which would no longer grow *in vivo*, unless very large numbers of tumor cells were implanted. The mutants had clearly become more immunogenic than the parent tumor. One of these tumor- (tum-) variant clones was used to immunize syngeneic mice. This generated T-cytotoxic (Tc) cells, which would kill only the immunizing tumor and not the parental line or other tumvariants (**Figure-6**). The Tc cells were then used as probes to identify the presence of the mutated tumor antigen during molecular cloning of the tumor antigen gene. Ultimately the mutant gene coding for the tumor antigen (the tum-gene) was identified and sequenced. Comparison of this gene with the homologous gene from the parental tumor showed a single amino acid difference. Formal proof that this mutation could generate the immunogenic antigen recognized by the Tc cells was then obtained: parental tumor cells incubated with a ten-amino-acid peptide having the tum-sequence could be killed, while if they were incubated with the homologous peptide from parental cells they were not (**Figure-7**). As Tc cells are MHC class I restricted, it is clear that the tumor-specific protein is processed within the cell to generate a peptide that becomes associated with MHC class I and is transported to the cell surface (**Figure-12**).

In contrast to the first tum- antigen, antigen genes cloned from other tum- variants were sometimes identical to the parental gene. In these tum- variants, the difference from the parent tumor was that the antigen was over-expressed in the cells.

There is good evidence that there are T-helper (TH) cell responses to tumors, but much less is known about the antigens recognized in association with MHC class II. This is because these antigens are normally recognized by TH cells on antigen-presenting cells rather than the tumor cell itself. This creates additional difficulties in cloning the genes coding for the antigens.

HUMAN TUMOR-ASSOCIATED ANTIGENS DETECTED BY IMMUNE CELLS

Mixed lymphocyte-tumor cultures reveal anti-tumor responses *in vitro*

The realization that responses of antigen-primed TH and Tc cells could be revealed by re-stimulating them with specific antigen in vitro led to experiments in which lymphocytes from patients were cultured with inactivated tumor cells in mixed lymphocyte-tumor culture (MLTC) (**Figure-8**). The lymphocytes might be taken from peripheral blood, from tumor-draining lymph nodes or from the tumor itself (the later are known as tumor-infiltrating lymphocytes or TILS).

MLTC may stimulate CD4 TH cells, which proliferate and secrete effector cytokines, and CD8 Tc. The latter can be assayed by measuring their cytotoxic activity in a ^{51}Cr-released assay. Tc cells can also be obtained from TILs by culturing them in IL-2 to expand any

effector cells generated in vivo. Tc clones obtained by these means have been used to identify human TSTA genes by similar molecular expression cloning techniques to those used for tum- variant genes in the mouse.

Melanoma appears to be particularly immunogenic and melanoma-specific CTL clones were therefore produced and used to screen a melanoma cDNA library. The first human tumor antigen identified, MAGE 1 (melanoma antigen 1), was an overexpressed unaltered antigen (**Figure-9**). More remarkably the antigen belongs to a large previously undiscovered gene family of cancer testis antigens, which are widely expressed in tumors and seldom in normal tissues. Subsequently several other melanoma antigens as well as antigens of other tumors have been cloned. The majority of antigens identified by this methodology are unaltered. Many are differentiation antigens, normal molecules that are expressed in a tissue-specific fashion and play a role in the function of the normal cellular counterpart of the tumor cell, in the case of melanoma, the melanocyte. However, mutations and genetic rearrangements creating new sequences are common in tumors (**Figure-10**) and some tumor patients can respond to peptides of mutant ras oncogene or the HER2/neu oncogene product. Computer analysis indicates that many of the new sequences formed by genetic alternations in tumors could bind to common HLA alleles, such as HLA-A2, and may therefore be potential tumor target antigens. These studies have also established the principle that most tumor antigens do not differ from other antigens recognized by T cells; they are short peptide presented by MHC molecules.

TUMOR-ASSOCIATED ANTIGENS DETECTED BY ANTIBODIES

Few antigens are unique to tumors

There have been many attempts to detect antigens unique to tumors, using either sera from animals deliberately immunized with tumor material (heterologous typing) or sera from tumor-bearing animals or patients (autologous typing). In recent years heterologous typing has relied on monoclonal antibodies (mAbs) and although few molecule uniquely expressed in tumors have been detected, several types of antigen associated with tumors have been identified (**Figure-11**).

Tumors may express normal differentiation antigens that have a restricted distribution in normal cells

Most tumor cells represent the clonal progeny of a single cell, and cells of that type may be relatively rare. The tumor cells may therefore express antigens present on only few normal cells. The Common Acute Lymphoblastic Leukaemia Angitgen (CALLA or CD10) is an example (**Figure-12**). Oncofetal antigens are differentiation antigens expressed during fetal development but normally not expressed, or expressed at very low levels, in adult life. Examples are α-fetoprotein (AFP), which is produced by liver cancer cells, and carcinoembryonic antigen (CEA) produced by colon cancer cells and other epithelial tumors.

Normal antigens expressed in tumors may be altered by glycosylation

Glycosylation is altered in many tumors. This may give rise to the expression of new carbohydrate epitopes, such as the Thomsen-Friedenreich antigen, a disaccharide which is usually hidden on normal cells. Aberrant blood groups can also be created in this way. Alternations in glycosylation may also reveal epitopes on the protein backbone that are rarely detected in normal cells. For example, polymorphic epithelial mucins are produced by many normal epithelial cells. They are high-molecular weight glycoproteins with a repeating core peptide carrying the carbohydrate side chains. In epithelial tumors, new peptide epitopescan be detected in the repeating core structure of polymorphic epithelial mucin 1 (MUC 1), but these new epitopes are also detectable in the lactating breast (**Figure-11**).

Sera from patients with tumors detect widely distributed antigens

Until recently autologous typing was extremely difficult because most human sera are complex and contain many anti-bodies capable of reaching to tumor cells including anti-HLA, Anti-blood group and anti-carbohydrate antibodies. Many antibodies are IgM and of low affinity. Generation of human monoclonal antibodies has been technically difficult and the resulting hybridoma antibodies often detect widely distributed autoantigens, perhaps because they are derived from the pool of natural autoantibody producing B1 cells. The importance of these antibodies in the host response to tumors is unclear.

Serological analysis of human tumor antigens by recombinant cDNA expression expression cloning

More recently SEREX has been developed. Sera from patients are used to screen cDNA expression libraries from fresh tumor material. Isolation of antigens detected only by high titre IgG or IgA antibodies ensues that the method does not detect IgM natural antibodies. The SEREX method has the disadvantage that it may not detect all conformational eqitopes of a protein and does not identify carbohydrate antigens because the bacteria used to express the antigens do not glycosylate them. Nevertheless, over 900 sequences of genes cloned using the SEREX method have already been deposited in a data base set up for this purpose. These include known TSTAs such as MAGE-1 and tyrosinase, sequences identical (or nearly identical) to known genes not previously known to elicit an autoantibody response (e.g. kinectin a transporter associated with Golgi vesicles), and a large group of previously unknown genes (**Figure-13**). A complete description of the expression patterns of 900 genes in normal and tumor tissue, let alone analysis of their functions, is a major undertaking, but will eventually identify many new targets for immunotherapy.

IMMUNODIAGNOSIS

Antigens need not be tumor specific to be used for diagnosis

Although there are few molecules which are exclusive to tumor cells, antibodies to tumor-associated molecules can be very useful in tumor diagnosis, by either detecting increased amounts of an antigen or the presence of an antigen in an abnormal site.

In vivo

Radio-labeled antibodies against tumor-associated molecules have been used for the detection of tumors (**Figure-14**), but the method is seldom more sensitive than modern methods of computerized tomography or nuclear magnetic resonance imaging. In addition, immunoscintigraphy has the disadvantage that antibodies need to be freshly labeled for each patient, ad different antibodies are optimal for different tumor types. The development of recombinant multivalent fragments of high-affinity antibodies may improve the sensitivity of immunoscintigraphy in the future.

In vitro

Antibodies are useful for identifying the cell of origin of undifferentiated tumors (**Figure-15**) and for the detection of micrometastases in bone marrow, cerebrospinal fluid, lymphoid organs or elsewhere (**Figure-16**). There are also immunoassays available for several tumorassociated molecules which can be detected in the serum. These include CAE, AEP and PSA. Raised levels of CEA or AFP may be useful for diagnosis but CEA my be raised in association with several tumor types and both CEA and AFP in some non-malignant conditions so that they are generally more useful in following the course of treatment (**Figure-17**).

IMMUNOTHERAPY

Immunotherapy has a limited role at present

Immunotherapy has along history but is only now becoming established as a reliable form of therapy for some forms of cancer, while most immunotherapeutic strategies remain experimental. Intervention may be active or passive, specific or non-specific. **Figure-18** summarizes the possibilities.

Specific active immunotherapy – the mechanisms of activation

Specific active immunization with inactivated tumor cells has shown some success in animal models where immunization is performed before tumor challenge. Attempts to induce regression of established tumors have been much less successful. While much effort has been expended in designing means of making tumor cells more immunogenic, for example by infecting the cells with viruses or coupling chemical group to the cell surface, most of these have been empirical. More recently it has become clear that induction of immune responses depends on two signals. Signal-1 is delivered through the T-cell receptor (TCR) when it interacts with MHC-peptide complexes and the second essential signal for activation (Signal-2) is delivered by several costimuli. These may be both cell surface molecules on antigen-presenting cells (APC) and soluble cytokines

(**Figure-19**). There is good evidence that T cells may be inactivated if one signal is delivered without the other.

Immunization with tumor antigens

Increased understanding of the mechanisms of T cell activatation has led to more rational strategies to improve the immunogenicity of tumor cells. Transfection of the genes for CD80 (B7) or cytokines such as interleukin-2 (IL-2), IL-4, interferon-γ (IFN-γ) or granulocyte-macrophage-colon-stimulating factor (GM-CSF) into tumor cells has been shown to increase greatly their immunogenicity in animal tumor protection experiments. Similarly, immunization with defined peptide epitopes in novel adjuvants can induce cytotoxic T lymphocyte (CTL) able to cause rejection of experimental tumors. DNA constructs coding for tumor antigens and co-stimuli can also be used directly to immunize animals. Heat-shock proteins extracted from tumor cells have also been used. It is thought that these molecules act as chaperonins during the assembly of MH-peptide complexes, carrying peptides produced during antigen processing. While all these methods can protect animals against subsequent tumor challenge, they are much less successful in treating established tumors.

Immunization with dendritic cells

A logical extension of attempts to make tumor cells more immunogenic by transfection of co-stimuli is, instead, to use the most effective APCs known. These are dendritic cells, which can be grown from the bone marrow of mice or peripheral blood of man. An additional advantage of this method is that it is though that even co-stimulus-transfected tumor cells seldom present antigen effectively to resisting T cells. Rather, dead or dying tumor cells are more usually taken up by APCs and carried to the draining lymph nodes, where activation of T cells takes place. Antigens can be introduced into dendritic cells *in vitro* as whole (irradiated) tumor cells or as proteins, peptides, DNA, or in recombinant viruses. The cells are then reintroduced into the tumor-bearing animal or patient (**Figure-19**). At least in experimental animals this method, though cumbersome, is showing promising results in tumor therapy experiments.

Active immunization in man

Although no form of active specific immunization has yet become a first-line treatment for any cancer, many clinical trials are currently underway (**Figure-20**). As both TH and Tc cells have been shown to contribute to tumor protection in animal experiments, not all the immunization procedures are aimed at the induction of cytotoxic T cells; for example, the use of oligosaccharides or lipids conjugated to carrier molecules such as Keyhole Limpet Haemocyanin (KLH) would be expected to induce TH and antibody production, but Tc.

In most trails, prolonged survival has been observed in some patients following immunization and in most cases side effects have been mild. Most trials to date have been

in patients who have relapsed after initial treatment by surgery, radiotherapy and chemotherapy. These patients would not be expected to be ideal subjects for immunotherapy as they have a high tumor burden, the tumor will have had time to develop escape mechanisms (see below) and radiotherapy and chemotherapy are immunosuppressive. Future trails will need to be carried out at an earlier stage of disease to test whether immunotherapy is a useful adjunct to standard treatments which have firs eliminated the main tumor burden.

Non-specific stimulation of immune responses

A variety of agents have been used to stimulate the immune response non-specifically (**Figure-21**). Most attempts at systemic therapy in man have not been conspicuously successful, but Intralesional BCG can cause regression of melanoma and non-specific local immunization with BCG is effective against bladder tumors.

Immunotherapy with cytokine can cause tumor regression

Many cytokines have been cloned, expressed and used for tumor therapy. **Figure-22** gives information on those that have been most thoroughly investigated to date. Success have so far been few and far between, though IFNα can induce prolonged remission of the rare hairy-cell leukaemia and IL-2 is effective in a proportion of melanomas and renal carcinomas. There are also encouraging results in the treatment of intraperitoneal ovarian tumors with IFNγ and tumor necrosis factor-α (TNFα). Some cytokines are finding a useful role in supportive therapy after bone marrow transplantation or cytotoxic therapy, and erythropoietin can relieve the anaema.

Immunization against oncogenic viruses

Because there is increasing evidence for a role of viruses in some human cancers, the most promising avenue for active immunization may be in preventing infection with potentially oncogenic agents. Successful mass immunization against hepatitis B virus is already decreasing the incidence of primary hepatoma in endemic areas. It may be possible eventually to vaccinate at-risk populations against papillomaviruses,HTLV-1 or EBV.

PASSIVE IMMUNOTHERAPY

Therapy with lymphokine-activated killer cells

When human peripheral blood mononuclear cells are cultured *in vitro* with IL-2, they become highly cytotoxic to a wide variety of tumor targets, many of which are resistant to freshly isolated natural killer (NK) cells. Initial animal and human experiments, in which these lymphokine-activated killer (LAK) cells were re-infused, gave some good

results, especially when IL-2 was given at the same time. However, controlled trials have given less encouraging results and the therapy, involving high-dose IL-2, has significant toxicity. It seems likely that few LAK cells localize in tumors, and this may contribute to the poor results. To overcome this, bi-specific mAbs have been used. In these, one antibody is directed against a tumor molecule and the other against lymphocyte surface markers. In theory these antibodies should help to localize the LAK cells on the tumor. While such strategies certainly work *in vitro*, their effectiveness *in vivo* remains to be established.

Immunotherapy with T cells

T cells extracted from tumor sites can also expanded in vitro using IL-2 and eventually re-infused. In a proportion of cases the cultured T cells show relative specificity for the tumor from which they were derived. In animal model systems there is no doubt that tumor-specific cytotoxic T cells can cause dramatic regression of tumor. The tumor toxicity of such TILs may be increased by transfecting into them genes coding for cytokine production. In humans large numbers of EBV-specific Tc cells have been grown in vitro using IL-2 and infused into patients who have developed lymphoma following bone marrow transplantation. Remission of tumor occurred. In this case the strong vital antigens of EBV are the target but although these results are encouraging, the efficacy of this strategy against common epithelial malignancies remains to be tested.

The efficacy of anti-tumor lymphocytes is also shown by the graft-versus-leukaemia effect. Following allogeneic bone marrow transplantation for leukaemia, it was noticed that patients who developed graft-versus-host disease had a better prognosis than those who did not. This led to the use of leucocyte transfusions from the donor of the bone marrow, in addition to the bone marrow transplant itself. This has been shown to the effective in chronic myeloid leukaemia. Attempts are being made to separate cells mediating the graft-verses-leukaemia effect from those causing graft-verses-host disease. The possibility of using allogeneic cells for treatment of other forms of cancer is being explored.

Therapy with antibodies

Early attempts at passive immunotherapy with polyclonal antisera were limited because of the difficulty of achieving high titre and specificity. The advent of monoclonal antibodies overcame these difficulties. Although, with the exception of B-cell and T-cell idiotypes on lymphomas, surface antigens unique to tumors have not been discovered, some antigens show increased expression on certain tumor cells and in other cases, damage to normal body cells carrying the same antigen may be unimportant or tolerable. Monoclonal antibodies may be used either alone, or coupled to drugs, pro-drugs, toxins, cytokines or isotopes (**Figure-23**). There are however, a number of limitations to antibody therapy:

- Antibody penetration into large tumor masses is often poor. In principle this might be overcome by smaller molecules that retain specific antigen binding, e.g. F(ab') fragments, or by engineered single-domain antibodies. Alternatively, it may be possible to target therapy to the endothelium of tumor blood vessels.

- Antibodies are bound by any normal cells expressing the target antigen, and non-specifically by cells bearing Fc receptors or receptors for immunoglobulin carbohydrates. Chemical modification or genetic engineering of the antibody molecules may partially overcome these difficulties. Better discrimination between tumor and normal cells might be obtained with bi-specific ant-bodies against two different antigens which are both present on the tumor cells but only found separately on normal cells.

- Antibodies are immunogenic and may therefore be attacked by the immune system. Even chimeric or humanized antibodies may induce an immune response to their idiotype. The use of different mAbs for successive courses of therapy might solve this problem.

In spite of these difficulties there have been some encouraging results. In a randomized study the 70-1A monoclonal antibody has been used to treat colon cancer following surgery to remove the primary tumor. Here the aim was to target micrometastases, avoiding the problem of poor penetration into large tumor masses. The treated group showed significantly improved survival. An antibody to the HER2/neu growth factor receptor has been licensed in the USA for treatment of breast cancers expressing the receptor. In this case the most promising results appear to be obtained in combination with chemotherapy. A monoclonal antibody to the CD20 B-lymphocyte differentiation antigen has also received a licence for treatment of lymphoma.

Raiolabelled anit-B-cell antibodies also show promise against lymphomas resistant to conventional therapy and a labeled monoclonal against the MHC-1 antigen appears to prolong survival (compared to historical controls), when administered intraperitoneally to patients with ovarian cancer.

Antibodies may also be used in vitro either to purge tumor cells from bone marrow for autografting (**Figure-24**) or to remove T cells for prevention of graft-versus-host disease in allotransplants.

IMMUNE ESCAPE MECHANISMS

Tumors shows multiple mechanisms for evading immune responses

Because spontaneous tumors grow and kill the host, many tumors must escape the host immune response. Many mechanisms have been proposed. The most obvious is that the tumor is non-immunogenic. This might be because potential tumor antigens are lacking

but, as described either, increasing numbers of antigens recognized by cells or antibodies of tumor bearers are now being identified. More likely the weak response to tumors is because they are poor antigen-presenting cells. Even if effector cells are generated, these may recognize (and kill) the tumor cells with difficulty.

A particular important escape mechanism is loss of MHC antigens leading to inability to present tumor antigen peptides. More than 50% of tumors may lose one or more HMC class-I alleles and sometimes all class-I (**Figure-25**). A variety of molecular mechanisms has been identified, including mutations in β-2 microglobulin and peptide transporters. The common occurrence of MHC loss in tumors strongly suggests that there is selection for it, presumably by cytotoxic T cells.

Induction of immune responses requires co-stimuli, as does optimal function of effector cells. the CD80 (b7) and CD40 molecules, present on specialized APC, are now known to be key co-stimuli acting via their counter-receptors CD28 and CD40L on the T-cell surface. Experimentally, presentation of MHC-peptide antigen complexes to the T cell receptor in the absence of CD80 co-stimulation may lead to anergy, and there is evidence that TILs may sometimes be anergic. This effect may be part of a more general defect in immune responsiveness in cancer patients, because even peripheral blood T cells of tumor patients frequently show defective T cell receptor signaling *in vitro.*

Tumor cells may also lack other molecules required for adhesion of lymphocytes such as LFA-1, LFA-3 or ICAM-1, or they may express molecules such as mucins, which can be anti-adhesive. They may also secrete immunosuppressive cytokines such as transforming growth factor-β (TGFβ) and vascular endothelial growth factor (VEGF).

Relative risk of tumours in immunosuppressed kidney transplant recipients

Tumour type	Approximate relative risk
Kaposi's sarcoma	50–100
Non-Hodgkin lymphoma	25–45
Carcinoma of the liver	20–35
Carcinoma of the skin	20–50
Carcinoma of the cervix	2.5–10
Melanoma	2.5–10
Lung	1–2

Figure-1 In all forms of immunodeficiency the relative risk of developing tumors in which viruses are known to play a role is greatly increased. This is the case for all those listed except cancer of lung. The relative risks vary in different studies according to the length of follow up and the presence of cofactors such as sunlight for skin cancer.

Tumour viruses and immunodeficiency

cause of immunodeficiency	common tumour types	viruses involved
inherited immunodeficiency	lymphoma	EBV
immunosuppression for organ transplants or due to AIDS	lymphoma	EBV
	cervical cancer	papilloma viruses
	skin cancer	probably papilloma viruses
	liver cancer	hepatitis B and C viruses
	Kaposi's sarcoma	human herpes virus 8
malaria	Burkitt's lymphoma	EBV
autoimmunity	lymphoma	EBV

Figure-2 In organ transplant recipients cancer of the sin is the most common form of tumor in absolute number. In other forms of immunodeficiency tumors of the immune system dominate. Most normal adults carry both EBV and many papilloma viruses throughout life with no ill effects because they have anti-viral immunity.

Role of EBV in tumorigenesis

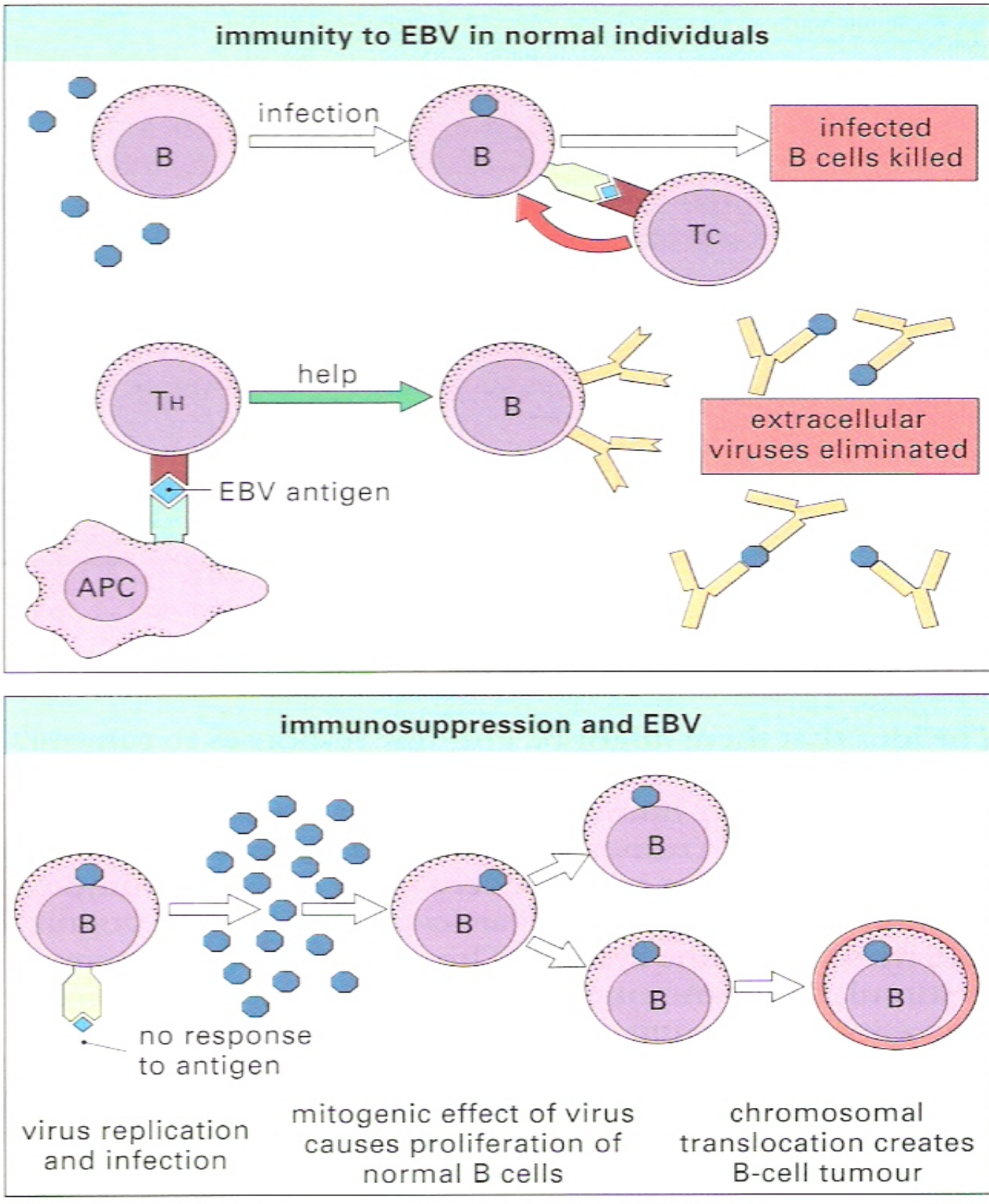

Figure-3 In normal individuals EBV infects B lymphocytes but spread of infection is prevented by Tc cells and antibody, which eliminate infected cells and virus. In immunosuppressed individuals, and in some patients receiving the immunosuppressant cyclosporine, the virus replicates and infects more B cells. The virus is also mitogenic for B cells, so in an immunosuppressed individual infected B cells tend to proliferate more rapidly. A chromosomal translocation in an infected B cell can then lead to malignant transformation.