TUMOUR AND TRANSPLANTATION IMMUNOLOGY

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Interest in the immune response developed from a study of the host's reaction to infection. Today the ramifications of immunology permeate a much broader span of medical practice. It is realized that selective suppression of the immune response may hold the key to the successful transplantation of tissues. In contrast, stimulation of the immune response may provide a means by which we can effect the rejection of cancers. This relationship between transplantation and tumour immunology is an important one and is the reason for inclusion of these two topics in this article.

Terminology

The immune response is the reaction of an individual to a substance or an organism which is foreign to it. The basic premise of tumour and transplantation immunology is that both grafts and tumours contain antigens foreign to the host. A clear understanding of the terminology that applies to tissue grafts is germane to both tumour and transplantation immunology:

A syngeneic graft is a graft exchanged between individuals of identical genetic constitution: for example, grafts between identical twins or mice of the same pure line strain.

An allogeneic graft is a graft exchanged between members of the same species but of different genetic constitution: for example, from one human to another or from one pure line strain of mice to a different one.

A xenogeneic graft is a graft exchanged between individuals of different species: for example, from monkey to man or mouse to elephant.

Tumour antigens

For a tumour to stimulate an immunological response it must possess characteristic antigens. Much of the early work on tumour antigenicity was confused because of the failure to appreciate the fact that tumours, like most other tissues, exhibit transplantation antigens. Only when syngeneic tumours are studied can consideration be given to those antigens that are characteristic of tumours. In animals this was possible after introduction of inbred mouse strains. Tumour-specific antigens are demonstrated experimentally when pretreatment with a syngeneic tumour influences growth if there is a subsequent challenge with the same tumour. Pretreatment may involve ligation or excision of the initial tumour after it has reached a critical size, but before dissemination (Klein et al., 1960), injection of a low dose of cells which is too low to induce an overt tumour, or injection of tumour cells that have been treated chemically or physically to prevent growth or division. If pretreatment alters the growth of an inoculation of tumour cells that would normally initiate an overt tumour in a non-treated recipient, then such pretreatment has caused an immune response to the characteristic antigens of the tumour. If pretreatment alters the growth of a tumour which is different from the one used for pretreatment, then cross-reactivity between the tumours used for pretreatment and challenge is said to occur. There are also in vitro techniques for detection of tumour antigens. Some involve the demonstration of tumour antibodies by membrane immunofluorescence, complement fixation or cytotoxicity, whilst others demonstrate lymphocyte cytotoxicity. Although antibodies to tumour antigens have been demonstrated by a variety of techniques, their significance in vivo is poorly understood.

It would appear that tumours may contain several antigens capable of stimulating an immune response: transplantation antigens, tumour-specific antigens, viral antigens, fetal antigens. Transplantation antigens are found on all tissues in vertebrates. Whether tumour-specific antigens are dependent upon new genetic information or whether they represent a de-repressed fetal gene has yet to be established. Viral and fetal antigens are usually cross-reacting. The antigens induced by both DNA and RNA viruses are the same for all tumours induced by a single virus,
irrespective of histological type, but differ from those induced by a different virus. The relationship between fetal and tumour antigens has been examined in a variety of ways (Stonehill and Bendich, 1970). Immunofluorescence studies have demonstrated fetal antigens on the surface of chemically induced tumours. Cytotoxic tests in vitro have demonstrated that lymphocytes from tumour bearers are sensitized against fetal antigens. In vivo most workers have found that pretreatment with fetal tissues protects against subsequent tumour challenge (Coggin, Ambrose and Anderson, 1971), but some have found that tumour growth is enhanced, and others have not found significant effects.

Demonstration of human tumour antigens depends upon in vitro techniques. Generally, human tumours contain common antigens shared by most, if not all, tumours of the same histological type. Cross-reactivity between tumours of different histological types is not usually found. This antigenic specificity is similar to that found in experimental tumours and it is evidence suggestive of a viral aetiology of most human tumours.

**Immunosurveillance**

The mechanisms whereby the host mounts a response against the antigens expressed by a tumour are known as immunosurveillance. It was stated by Burnet (1970) thus, "A major function of the immunological mechanism is to recognise and eliminate foreign patterns of behaviour arising in the body by somatic limitation or some equivalent process." This concept suggests that a mutant cell, which is potentially responsible for overt tumour development, has at least one antigen with a biochemical sequence different from that normally found in the host. An immunological response is, therefore, mounted against this antigen and may lead to the appearance of a clone of immunologically competent cells which eliminate the abnormal mutants. Immunosurveillance is an intellectually satisfying thesis which of its very nature is not open to direct testing. There is general agreement that some form of surveillance occurs continuously, but the points of debate are whether immunological rejection is necessary, rather than elimination by non-immunological mechanisms, and whether it has specificity. There is now evidence both for and against these contentions. Recently Prehn (1972) has suggested that stimulation of the immune response may encourage tumours to develop. It may be that several types of surveillance against tumours are operational and only one is mediated through the immune system.

**Escape from surveillance**

That tumours develop in animals and man and grow progressively and kill the host is an all too common observation in clinical practice. If immunosurveillance exists there must be escape mechanisms which protect tumour cells:

- Tumour antigenicity;
- immunoresistance;
- sneaking through;
- vascularization;
- genetic factors;
- immunosuppressants;
- anergy;
- blocking factors.

Tumours which arise spontaneously seem to be considerably less antigenic than induced ones. Immunosurveillance may be highly efficient in destroying antigenic tumours but may be ineffective against other tumours. Immunoresistance may develop when antigenic autochthonous tumours are exposed to immunological reactions that do not entirely eliminate them. The situation is analogous to bacteria developing resistance to antibiotics. The concept of sneaking through is based on the observations of Old and others (1962). They found that medium-sized inocula of antigenic tumour cells fail to grow in a sensitized animal, but a large dose of tumour cells overwhelms the immune mechanism and grows progressively. Furthermore, a small number of cells can also grow to an irreversible tumour colony before an immune reaction is mounted and this is "sneaking through". It may be that vascularization is the time when the nascent tumour colony becomes invulnerable to immunological attack. Tumour vascularization occurs by an ingrowth of host vascular channels into the tumour and, once established, rapid growth of the tumour occurs (Folkman, 1978).

The successful escape of tumours may result from changes in the host. The response to various antigens, including tumour antigens, can be genetically determined. Exogenous administration of immunosuppressive drugs can diminish host immune response and increase tumour incidence (Penn, 1975). However, patients with tumours show a non-specific depression of immune responses or anergy. The mechanisms causing this are not clearly understood, but may include suppressor lymphocytes and circulating serum factors (blocking factors), which inhibit the usual interaction of host defenses and tumour cells.

**Tumour destruction**

Tumour immunity is frequently assumed to be a variant of that found in transplantation and, there-
Therefore, mediated by the same mechanisms. However, the immunological mechanisms of tumour cell destruction are not completely known and there is disagreement about the relative roles of the separate components of the immunological apparatus in this process. It has not been possible to implicate any one cytotoxic mechanism in the immune elimination of tumours and different mechanisms seem to dominate in varying situations. The importance of T lymphocytes is well established, but there is considerable evidence for other cytotoxic cells (Woodruff, Dunbar and Ghaffar, 1973). One such mechanism comprises cells which have no direct affinity for target cell antigens, but are triggered to kill by antigens bound to the target cells. The cells involved in such antibody-dependent cytotoxicity (ADCC) are not clear. Antibody (from B lymphocytes) may be involved alone or in the presence of complement for tumour cell destruction. Another type of serum that promotes cytotoxicity has been termed unblocking serum by Hellstrom and colleagues (1971), and it is thought to represent the interaction of free antibody with blocking complexes.

The role of phagocytes in tumour destruction is not clear, but resistance of animals to tumour parallels reticuloendothelial phagocytic activity. Although stimulation of reticuloendothelial function protects against some tumours the biological mechanisms underlying these observations are not clear. Macrophages certainly exert a critical role initiating and maintaining immune reactions (Feldman and Palmer, 1971). Apart from processing antigen for lymphoid cells, phagocytic cells may be a significant effector mechanism in tumour destruction (Evans and Alexander, 1972). Phagocytic cells have other functions that may be important for control of tumours: they are essential for the response of lymphocytes to some mitogens and, by removal of the blocking factors from the circulation, they may play an important role in restoring the balance between host defences and tumour cells.

**Immunotherapy**

Immunoprophylaxis is the induction of resistance to tumour before its development and immunotherapy is the treatment of established tumours by immunological methods. In laboratory animals immunoprophylaxis has been encouraging. In humans this has not been practised in the true sense, although Rosenthal and colleagues (1972) showed that vaccination with B.C.G. protected children against the subsequent development of leukaemia. When immunological treatments are used as an adjuvant to conventional methods such as surgery, radiotherapy or chemotherapy to prevent recurrent disease, then immunoprophylaxis and immunotherapy merge imperceptibly.

Immunotherapy results have been disappointing. Treatment may be specific, designed to cope with a particular tumour, or non-specific, when the overall immunological reactivity of the host is changed. Specific immunotherapy can be passive, adoptive or active. Passive therapy involves the use of antisera which may be syngeneic, allogeneic or xenogeneic and then specifically absorbed. Adoptive therapy necessitates the transfer of syngeneic, allogeneic or xenogeneic lymphoid cells from specifically immunized donors. Active immunotherapy aims at increasing the immunogenicity of tumour cell antigens which are weak. Tumour cell antigens can be rendered more immunogenic by changing the dose or route of administration and by alteration of the cells in a variety of ways: physical treatments; viral incorporation; chemical modification; surface changes by enzymes; coupling with immunogens.

Non-specific immunotherapy involves a general increase of immune reactivity. With present knowledge, four methods of stimulation of host responses are available which might retard tumour growth:

1. Increased or improved localization of cytotoxic antibody.
2. Suppression of blocking factors.
4. Improved cell-mediated immunity.

A combination of specific and non-specific therapy has been the commonest approach to immunotherapy in clinical practice (Mathe, 1971). The rationale for the treatment is that non-specific adjuvants will generally boost the immune response including any reaction to synchronously administered exogenous tumour antigens.

Immunotherapy of human tumours has a long and undistinguished history. The majority of studies have so far been uncontrolled.

**Allograft rejection**

At one time it was thought that the immunological mechanisms involved in tumour cell rejection were similar to those involved in organ graft rejection. Although some mechanism may operate in both situations, it is unlikely that the processes are analogous in detail.
The cellular basis of immunological reactions to allografts is best worked out in the case of skin grafts in animals when genetically defined models can be used. The process has an afferent pathway which involves sensitization of lymphocytes by contact with the graft or, alternatively, soluble graft products reach the draining lymph nodes. It has been established that the integrity of the regional lymphatics is an important requirement for the afferent pathway of graft sensitization, although lymphatics do not seem to be required for rejection. In the central phase, sensitized lymphocytes reach the regional nodes and proliferate. The population of cells sensitive to skin allografts belongs to the recirculatory lymphocyte pool and a specially sensitized population probably results from proliferation at first in the regional node, which is then continued in the more distant nodes. In the efferent phase, cells of the recirculating pool, many of them newly formed by proliferation either in the lymph nodes or bone marrow, subsequently reach the allograft. At that site there is initial massive recruitment of normal unsensitized B and T cells by the sensitized cells. Secondly, there is an attraction of macrophages into the area, probably as a result of the release of macrophage inhibition factor by sensitized lymphocytes.

After kidney grafting it is the combination of antibody acting on the vasculature of the kidney, together with cellular infiltration, which produces the characteristic histological and pathological features of hyperacute, acute and chronic rejection.

Hyperacute rejection is the counterpart of the secondary immune response and it occurs in patients who have already been sensitized to specific donor antigens. In extreme cases the kidney becomes cyanosed shortly after implantation, even on the operating table. Histologically, there is thrombosis of the medium and smaller arterioles with parenchymal haemorrhage and cellular infiltration. Acute rejection is characterized by a diffuse infiltration of the entire parenchyma by small and large lymphocytes, macrophages and small numbers of plasma cells. There is an especially heavy infiltrate around the smaller afferent arterioles. Chronic rejection may occur over a long period of time and it is characterized by progressive deterioration in renal function. There is progressive fibrosis and narrowing of vessels as a result of fibrin deposition.

Clinical renal transplantation

Attempts to transplant the kidney experimentally began in earnest in 1902, when Ullman reported the first successful experimental organ transfer using Payr's cannulae to achieve vessel anastomosis. The same year, Carrel described his "vascular patch" which, since that time, with some minor modifications, has been used for revascularizing organ transplants. In 1905, Floresco grafted kidneys orthotopically and subsequently he joined them to the femoral vessels and obtained good urine flow through a cutaneous fistula. In 1955, nine kidney transplants were reported between non-identical donors and recipients (Hume et al., 1955). Despite the lack of tissue-typing and immunosuppression, four of these grafts functioned for between 30 and 120 days. The original technique in humans was to anastomose the donor renal vessels to the femoral vasculature and to bring the ureter as a cutaneous ureterostomy.

In parallel with the development of techniques for vascular anastomosis and transfer of organs, there was rapid development of transplantation immunology. A new era followed the experiments of Medawar and his associates, who clarified the mechanisms of immunological rejection. It was not long before irradiation and antimetabolites, which showed promise in animal experiments, were applied to man to reduce rejection. In 1959, whole body irradiation to a recipient afforded the first prolonged survival of a kidney allograft.

Clinical renal transplantation is now an accepted and standard form of treatment for patients with chronic renal failure. Since 1963, 25 108 transplants have been registered with the American College of Surgeons–National Institutes of Health registry. In the United Kingdom 600 transplants are undertaken annually. Of transplants carried out in this country and Europe, 10% are from living related donors. In the United States this figure approaches 40–50%, although the introduction of health care programmes has increased the number of cadaver transplants. In contrast, in Australia only 2% of transplants are from living related donors.

Most rejection episodes and most recipient deaths occur in the first 2 years after transplantation, although later complications do occur. Results from the transplant registry show 2-year survival with a functioning transplant to be 74% after a sibling kidney transplant, 68% after receiving a kidney from a parent and 47% after cadaver kidney transplant. Individual units, with wide experience, or rigid criteria for accepting patients, or insistence on using only "beating heart" cadaver kidneys, report better results. Patient survival is considerably better and the corresponding survivals at 2 years are 83%, 81% and...
During the past few years, functioning kidney survival has not improved and this reflects the lack of progress in overcoming rejection. Despite this, results are comparable to, or better than, those obtained in the treatment of many of the major cancers and, moreover, many of the patients are young adults with dependents. In contrast, patient survival has consistently improved, probably because of the early abandonment of failing transplants, before the effects of excessive immunosuppression supervene. This policy is encouraged by the good results of second and subsequent transplants.

With increasing experience, transplantation is being offered to a wider group of patients with renal failure. Transplantation in patients with metabolic and systemic diseases is increasingly common and the recent reports for diabetic patients show that the procedure is worthwhile in this group (Najarian et al., 1973). Certain groups of patients have a better prognosis: for example, those with renal cystic disease. Although some diseases recur in the transplant (for example amyloid and dense deposit disease), they do not necessarily impair renal function significantly.

The full potential of transplantation surgery is at present hampered by the lack of donor organs. There are 1200 patients (the majority between 30 and 50 years) on file awaiting transplantation and it is estimated that 2500 patients could benefit from transplantation annually. Each year 600 kidneys are transplanted in Britain. Even a proportion of the annual 6000 fatal road traffic accidents, let alone those patients dying of cerebrovascular disease or cerebral tumours, would totally satisfy the transplantation needs of the country.

Because of the shortage of kidneys, there is no doubt that units use kidneys which, in a more affluent situation, might have been turned down. It has been shown that 17% of kidneys transplanted in this country never function, many because of excessive warm ischaemia (Nelson and Tovey, 1974). In the United States and some parts of Europe, primary failure as a result of ischaemic damage is very rare, since the public has accepted the concept of brain death and most kidneys come from patients with irreversible brain damage but with an intact circulation.

Whilst the survival of patients receiving cadaver renal transplants has steadily and significantly improved, functioning-graft survival has not changed for several years. This results from the high frequency of rejection and the lack of any significant change in immunosuppressive regimes for the past decade. Prednisone and azathioprine, with all of their attendant problems, are still the cornerstones of immunosuppression. In experimental studies heterologous antilymphocyte serum (ALS) is the most effective agent for the abrogation of cellular immunity. The remarkable ability of ALS to suppress allograft rejection in both small and large animals has generated much enthusiasm for its use in clinical transplantation. Unfortunately, despite its use for several years as an adjuvant immunosuppressive, its clinical effectiveness is still not established. In the United States, of 100 major centres, 67% used it and half of these thought it to be beneficial (Monaco, Campion and Kaprick, 1977). The few controlled trials of the agent have given equivocal results (Launois et al., 1977).

Other agents also have been studied recently. The importance of vascular changes in graft rejection is well established and agents affecting platelet function have been investigated. In experimental allografts, sodium salicylate, acetylsalicylic acid, heparin and dicoumarol all prolong graft survival. In clinical studies, dipyrimadole improved vascular lesions, but did not improve results. Cyproheptadine is a safe antihistamine with antiserotonin activity and appears to be a useful drug when used in combination with conventional treatment (Rattazzi, Simmons and Najarian, 1977). The antihelminth, niridazole, has been shown to prolong a skin allograft survival in rodents and it is immunosuppressive in man. However, side-effects are common and current studies are directed to finding a non-toxic, immunosuppressive metabolite (Salaman et al., 1977). Cyclosporin A is a new immunosuppressive which requires further clinical evaluation.

Because of the complications of pharmacological immunosuppression, the search for a specific form of allogeneic unresponsiveness that does not interfere with the remainder of the host's immunological defences is the central goal of transplantation research. The dividing lines between classical tolerance, enhancement and blocking have become increasingly tenuous. Although the classical experiments of Billingham, Brent and Medawar (1955) on neonatal tolerance demonstrated the principles involved, approaches which may be more clinically relevant utilize radiation and bone-marrow transplantation. Other approaches to specific unresponsiveness involve the use of antisera against
tissue incompatibility antigens, but more experimental work is needed before these techniques can be applied routinely to clinical practice.

Inadvertently, clinicians may have stimulated a degree of unresponsiveness by the use of blood transfusions. Originally it was feared that transfusion might presensitize patients and so decrease the likelihood of a successful subsequent transplant, for it has been shown that sensitized patients have a distinctly lower transplant survival rate (17% at 1 year) than those who are not sensitized (59% at 1 year). However, Opelz, Sengar and Mickey (1973) have suggested that transplant recipients who have received blood transfusions have a better chance of successful grafting than those who have never been transfused. More recently, Fuller and colleagues (1977) have shown that frozen blood is just as effective in promoting graft survival, but the frequency of sensitization is only 4.8% compared with 50% after whole blood. When the beneficial effects of transfusion and the detrimental effects of sensitization are combined, the theoretical chances of a successful graft at 1 year are 27% for those receiving no blood, 34% for those given whole blood and 57% after frozen blood. Further studies to determine the effects of different numbers of transfusions and the timing and relationship to grafting are required.

The mechanisms involved in these observations are not clear. Originally it was thought that some individuals are indiscriminate responders and others non-responders and transfusion was acting as a sorting mechanism to identify these groups. The alternative possibility of specific immunological enhancement is more attractive.

Immunogenetics

The immunological reaction to transplants is directed against genetically determined foreign substances, probably glycoproteins, on the surfaces of transplanted cells. Apart from ABO antigens, which may act as strong transplantation antigens, the most important of these substances are antigens of the major histocompatibility system (MHS) which are coded for in the MHS region (fig. 1). In this region there are three subloci which code for three serologically determined, SD (formerly HLA, Human Leukocyte Antigen) antigens and at least two lymphocyte determined, LD (formerly MLC, mixed lymphocyte culture) antigens. Serologically determined antigens are defined by sera from sensitized patients. By exhaustive testing of panels of antisera against panels of normal lymphocytes, using cytotoxicity or agglutination assays, it has been possible to develop patterns of specificity and gene frequency. LD antigens stimulate T cells in MLC but detection of surface immunofluorescence may aid direct typing for these specificities.

It is known that tumour allograft and probably organic graft rejection require interaction of both LD and SD antigens with both T and B cells. It is thought that T cells are stimulated primarily by LD antigens. In contrast, B cell antibodies, B cell cytotoxicity and ADCC are directed against SD determinants. In graft rejection the usual sequence of rejection is that T cells are stimulated by LD, then stimulated T cells together with B cells programmed to react against SD determinants interact to cause these cells to mount a reaction against SD.

Clinical histocompatibility testing

The relative influences of SD and LD compatibility matching on the ultimate outcome of renal allotransplantation is currently under investigation (Sachs, 1977). There is some suggestion that in patients matched for LD antigens, a mismatch for SD antigens may be important only if the patient has already been sensitized to SD antigens. Although the presence of MHS compatibility is important for graft survival, it is interesting that skin graft survival in SD identical members of the same family survive much better than SD identical individuals from different families. This suggests that there may be many minor determinants of histocompatibility, not coded for by MHS region, which are also important for graft rejection.

Recently there has been observation of an association between the MHS and disease susceptibility. During the past 10 years, about 40 diseases have been reported to have their susceptibility associated with or linked to tissue type; some of these are shown in table I. In establishing these associations, several
**Table I. Diseases having susceptibility associated with or linked to tissue type**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Antigen</th>
<th>Antigen frequency (%)</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankylosing spondylitis</td>
<td>B27</td>
<td>90/8</td>
<td>88</td>
</tr>
<tr>
<td>Caucasoid</td>
<td>B27</td>
<td>100/51</td>
<td>34</td>
</tr>
<tr>
<td>Japanese</td>
<td>B27</td>
<td>77/0</td>
<td>306</td>
</tr>
<tr>
<td>Reiter's syndrome</td>
<td>B27</td>
<td>78/8</td>
<td>36</td>
</tr>
<tr>
<td>Post-salmonella arthritis</td>
<td>B27</td>
<td>67/9</td>
<td>18</td>
</tr>
<tr>
<td>Post-Yersinia arthritis</td>
<td>B27</td>
<td>79/9</td>
<td>24</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Dw4</td>
<td>36/16</td>
<td>3</td>
</tr>
<tr>
<td>Addison's disease</td>
<td>B8</td>
<td>50/23</td>
<td>4</td>
</tr>
<tr>
<td>Haemachromatosis</td>
<td>Dw3</td>
<td>70/15</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>78/27</td>
<td>10</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>B14</td>
<td>26/3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>36/26</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>B7</td>
<td>34/25</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Dw2</td>
<td>67/18</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Group 4 (B cell)</td>
<td>84/33</td>
<td>11</td>
</tr>
<tr>
<td>Diabetes mellitus (juvenile onset)</td>
<td>B8</td>
<td>37/22</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bw15</td>
<td>23/15</td>
<td>2</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>B8</td>
<td>44/20</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>B8/B8</td>
<td>29/3</td>
<td>13</td>
</tr>
<tr>
<td>Graves' disease</td>
<td>B8</td>
<td>42/24</td>
<td>3</td>
</tr>
<tr>
<td>Caucasoid</td>
<td>Bw35</td>
<td>57/21</td>
<td>5</td>
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<tr>
<td>Japanese</td>
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<td>15/5</td>
<td>4</td>
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<tr>
<td>Psoriasis nonspecifica</td>
<td>Bw16</td>
<td>16/3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Bw17</td>
<td>28/7</td>
<td>5</td>
</tr>
<tr>
<td>Acute lymphocytic leukaemia</td>
<td>A2</td>
<td>53/44</td>
<td></td>
</tr>
<tr>
<td>Newly diagnosed</td>
<td>A2</td>
<td>83/44</td>
<td></td>
</tr>
<tr>
<td>&gt; 1500 days survivors</td>
<td></td>
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</table>

**principles are important. First, because antigen frequencies depend upon ethnic background, there must be adequately matched controls. In diagnosis, subgroups may be important. For example, there is no association between diabetes and tissue type, but if juvenile onset, insulin-dependent diabetes only is considered, an association is detected. Statistical analysis is imperative, and in this respect the association of a disease with one of 20 antigens will occur by chance alone. A distinction must also be made between association and linkage. Association is demonstrated in population studies of unrelated individuals when two particular traits occur together at a frequency different from that predicted by chance alone. Linkage cannot be proven by population studies, but must be shown in studies in families in which joint segregation of two traits can be demonstrated in a consistent pattern. The intensity of an association is measured by the relative risk (RR):**

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RR = \frac{\text{number of patients negative for the antigen}}{\text{number of controls positive for the antigen}}
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**REFERENCES**


