easily appreciated in examining a CSF without polymorphs. The same considerations apply to post-mortem examination where both macroscopic evidence, such as a septic spleen, and microscopic evidence in the form of leukocyte infiltration may disguise the true state of affairs at death.

Table 2 shows an analysis of causes of death in 46 patients treated with myelosuppressive agents or having primary bone marrow aplasia. These figures are influenced by many factors including the attitude of mind of the physicians concerned. Most of these patients, in fact, died after antineoplastic treatment had been abandoned. One hæmorrhagic death four years ago in a case of leukæmia shortly after admission might nowadays have been prevented. Another death, in which hæmorrhage played a part, resulted from the too-successful treatment of multiple metastases which simultaneously underwent hæmorrhagic necrosis, coincident with only a moderate degree of thrombocytopenia.

It is worth noting that among the cases in children were two virus infections, varicella and generalized herpes, but that at this age bacterial septicæmia was less common. Reluctance to perform blood cultures does not wholly account for the difference between children and adults. There was at least one mystery death, a 6-year-old child who developed convulsions during a phase of leukopenia. No bacterial, toxic or biochemical cause was evident and a careful post-mortem showed no cerebral or other lesion. An overwhelming virus infection seems the most likely cause.

Gram-negative bacilli were grown in all but one of the cases of fatal septicæmia. Except for one pseudomonas these organisms were probably endogenous rather than acquired. The co-existence of two or more organisms in 4 cases raises problems of treatment. If a narrow-spectrum antibiotic is used against a single sensitive organism, such as a staphylococcus, this may be rapidly replaced by a Gram-negative bacterium or by candida. Thus there is a strong argument for using broad-spectrum antibiotics, with or without antifungal agents, whenever serious infection is clinically diagnosed, and also for the prophylactic use of unabsorbable antibiotics and anti-fungal agents. The difficulties are that the antibiotics may be poorly tolerated or may be absorbed in dangerous degree from ulcerated areas in the intestine; they do not eradicate potential pathogens especially from areas such as the perineum or from pre-existing pockets of local infection, and they may even encourage the proliferation of resistant organisms, thus creating a new hazard. The problem requires full clinical trial.

An alternative or adjuvant method of treatment is the provision of fresh white cells which in practice involves the use of a blood cell separator. From a normal donor it is only possible to remove about 2×10^9 cells at a time and the benefit is probably only marginal although there may be circumstances, especially perhaps in children, where even this small number of cells may be valuable. In general, however, donors with chronic granulocytic leukæmia (CGL) are preferred because of the greater cell yield ($c.10^{11}$ cells) and because many of the cells continue to proliferate in the circulation and bone marrow for days or even weeks. There is a slight risk that the disease of CGL will be conveyed to the recipient and a rather greater hazard of graft-against-host reactions. These are mediated by the viable lymphocytes which may also be present in platelet concentrates; the risk is in proportion to the state of immunodepression of the recipient, the number of viable lymphocytes transferred, and the histocompatibility relationship of host and donor. A full scale graft-against-host reaction is probably not to be expected but the chance of impairment of bone marrow and lymphoid function by a minor reaction cannot be ignored. Irradiation of all cell suspensions for cases at risk is a simple and effective preventive measure. It is even possible to irradiate cells in circuit between donor and recipient and it can be shown that such an irradiation to a dose of 1,500 rad does not impair the viability of the granulocytes and even permits some continued proliferation of myelocytes.

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Tumour Development Following Immunosuppression

It is now generally accepted that most tumours in experimental animals have new antigens which are not present in the host tissues and are therefore able to elicit an immune response. As a rule, all tumours induced by a particular virus have common antigens, so that immunization with an oncogenic virus will protect animals against transplants of syngeneic tumours induced by the same virus, but not against tumours induced by other viruses.

In contrast, the tumours induced by chemicals usually have individually specific antigens, so that immunization by one tumour, with small doses of viable cells or larger doses of irradiated cells, will protect animals against transplants of that particular tumour line, but not against other tumours induced by the same chemical. This generalization requires some qualification; it now appears that tumours induced by the mammary tumour virus may have individually specific antigens in addition to the common antigen due to the virus (Morton *et al.* 1969); and some common antigens are observed in chemically induced tumours (Reiner & Southam 1969).

The observations on the presence of tumour-specific antigens are well established and there is no need to quote the extensive literature on the subject. Perhaps attention can be drawn to the work of Rapp's group on tumours in guinea-pigs, in which cell-mediated immunity can readily be studied (Churchill *et al.* 1968). This supports previous conclusions that the most effective responses against most tumours are cell mediated, and that in general humoral antibodies enhance rather than suppress tumour formation (Hellström *et al.* 1969).

When attempting to analyse the effectiveness of the immune response of the original host against its own tumour, immunosuppression provides a useful tool. Under a variety of conditions immunosuppression will increase the number of tumours induced by a virus or chemical. Such effects are particularly striking with virus-induced tumours in adult animals. An example is the finding of Gaugas *et al.* (1969) that CBA mice, thymectomized when 6 weeks old, infected with *Mycobacterium leprae* and treated with antilymphocytic serum, all developed tumours. No tumours were seen in infected mice not receiving immunosuppressive treatment. The tumours (mammary adenocarcinomas, mixed salivary gland tumours and osteosarcomas) were typical of those induced by polyoma virus, and the isolation of this virus from tumours, and the presence of antibodies in all the mice, whether tumour-bearing or not, suggested that a room infection with polyoma virus was responsible for the epidemic of tumours.

Since the original report, another similar experiment has produced identical results, details of which will be published elsewhere. Together with previous observations of tumours in neonatally thymectomized mice later becoming infected with polyoma virus (Law 1966, Allison & Taylor 1967), these observations show that the main reason for the resistance of adult mice to oncogenesis by polyoma virus is not failure of transformation but the efficiency of the immune response against virus-transformed antigenic tumour cells.

The experiments already performed were complicated by two factors: the presence of mycobacteria, which might have exerted some non-specific adjuvant effect, and the unknown time of exposure to the virus. It was therefore decided to repeat the experiment. Adult CBA mice were infected with polyoma virus in the absence of mycobacteria and the effects of restoration of immunity with specifically sensitized and non-sensitized syngeneic lymphoid cells were examined.

Virgin female mice of the CBA strain were thymectomized at 6 weeks and given weekly subcutaneous injections of 0·4 ml rabbit anti-lymphocytic globulin (ALG). Control mice were given normal rabbit globulin (NRG). Ten days after the inception of ALG or NRG treatment, mice were given intraperitoneal injections of 10^5 TCD 50 polyoma virus. ALG treatment was continued for seven weeks, at which time the first tumour appeared. At the eighth week the mice were divided into three groups. One was untreated, the second was given spleen cells from normal adult CBA mice (10^6 cells per gram weight of recipient intravenously) and the third was given the same number of spleen cells from adult CBA mice infected three weeks previously with polyoma virus. No further ALG or NRG was given.

The results are shown in Table 1. Before infection none of the mice had antibodies against polyoma virus. All sera tested at 8 weeks in all groups showed hæmagglutinin-inhibiting antibodies against polyoma virus of comparable titre. All of the thymectomized and ALG-treated mice with no restoration, and all but one of the mice restored with normal lymphoid cells, showed tumours. All but two of the tumours were mammary adenocarcinomas; two were mixed salivary tumours. None of the mice receiving sensitized lymphoid cells has developed a tumour during 8 months of observation.

Table 1

Development of tumours in adult mice infected with polyoma virus

Preliminary treatment	*Restoration at 7 weeks*	*No. of animals*	*Percentage developing tumours*
NRG	None	24	0
Thymectomy +ALG	None	14	100
Thymectomy +ALG	Normal lymphoid cells	10	90
Thymectomy +ALG	Sensitized lymphoid cells	11	0

To examine further the effects of restoration of cell-mediated immunity, 18 mice with small mammary tumours about the size of a pea were taken and the tumours excised under ether anæsthesia. Half the mice were given 5×10^6 polyoma-sensitized syngeneic spleen cells intravenously, and the others left alone. All the mice in the latter group developed tumours, due either to recurrence or separate origin. Only 1 of the 9 mice receiving sensitized lymphoid cells has developed a tumour.

These results demonstrate that the main factor allowing polyoma virus oncogenesis in the thymectomized ALG-treated adult mouse is suppression of cell-mediated immunity. The effect can be reversed by restoration with specifically sensitized lymphoid cells. The failure of restoration by normal lymphoid cells is presumably an effect of timing and parallels previous experience of restoration after neonatal thymectomy (Allison 1970). The normal lymphoid cells prevent tumour formation only when transferred one week after virus inoculation, whereas sensitized cells are effective when transferred one month after virus inoculation. The prevention of the reappearance of tumours after excision by transfers of sensitized lymphoid cells shows that under optimal conditions immunotherapy can be a remarkably effective adjunct to surgery.

Another lesson from observations in experimental animals is that virus-induced tumour cells can be suppressed by an immune response without being totally eliminated. They do not emerge as overt tumours, but persist for long periods, after which some will emerge spontaneously. If such carrier animals are treated with immunosuppressants, overt tumours appear in greater numbers and much sooner than in control, untreated animals. Since this situation may be relevant to what occurs in man, two examples will be described. One is the inoculation of vacuolating virus (SV_{40}) into adult hamsters (Allison *et al.* 1967). After a very long delay representing the greater part of the life span of the animals, about one-fifth of the hamsters develop SV_{40} tumours at the sites of inoculation. The incidence of tumours is higher, and the latent period is shorter, in X-irradiated recipients. The second example comes from recent collaborative work with Dr E F Wheelock, who had previously shown that pretreatment of DBA/2 mice with the interferon-inducer Statolon protects most mice against the early leukæmogenic effects of Friend virus; the survivors are 'carriers', and many develop late leukæmia after a delay of at least several months (Wheelock *et al.* 1969). We have observed that if such carrier animals are treated with ALG all develop frank leukæmia within one month. Hence the carrier state is an equilibrium in which potentially malignant cells and immune cells are both present; if the effectiveness of the latter is reduced by immunosuppression the tumour cells rapidly emerge.

Other relevant observations include potentiation of polyoma and Moloney virus leukæmogenesis in mice by ALS (Allison & Law 1968), potentiation of the naturally occurring pleomorphic reticulum cell sarcomas in SJL/J mice by ALG (Burstein & Allison 1970) and of reticulum cell sarcomas in NZB mice by azathioprine (Casey 1968). The tumours that have so far appeared in chronically immunosuppressed animals have either been typical polyoma tumours, confirmed by virus isolation and serology, or lymphomas in which there is reason to believe that an oncogenic virus is involved. There is not yet any clear evidence for appearance of tumours other than those of viral origin.

There is thus good evidence for the effectiveness of the immunological surveillance mechanism in limiting tumour development in adult animals, and for the breakdown of the surveillance mechanism following immunosuppression.

Does this have any relevance to human medicine? During the past two years there have been several reports of patients developing primary tumours while on immunosuppressive treatment for renal transplants (Doak *et al.* 1968, Zukoski *et al.* 1968, Woodruff 1969, Deodhar *et al.* 1969, Penn *et al.* 1969, McKhann 1969). These are in addition to patients developing tumours after transplantation of kidneys from tumour-bearing donors, in which the tumours were probably transferred with the kidneys (Martin *et al.* 1965, McPhaul & McIntosh 1965, Muiznieks *et al.* 1968, Wilson *et al.* 1968, Zukoski *et al.* 1968). At least 19 primary tumours in immunosuppressed subjects are now on record, 10 affecting the lymphoreticular system and presenting mostly as reticulum cell sarcomas.

Since the overall incidence of reticulum cell sarcoma is of the order of 1 in 100,000 (McKhann 1969), the increased incidence in the immunosuppressed subjects (numbering about 2,000 in all) is highly significant statistically. The overall incidence of tumours other than lymphoreticular is also significantly higher than expected in the relevant age group (8·2 per 100,000); the great majority of the patients were between 20 and 40. However, this group of tumours is heterogeneous, and as yet the incidence of no single tumour type is significantly raised. Nor is there a clear indica-

tion that any immunosuppressive procedure is more likely than any other to result in tumour formation. All patients received azathioprine and steroids; some had in addition thymectomy, splenectomy, irradiation, actinomycin D and/or ALS.

The other observation of interest is that children with immune deficiency syndromes have a much higher probability than other children of developing malignancy, again chiefly but not exclusively of the lymphoreticular systems. This is true of ataxia-telangiectasia, the Wiskott-Aldrich syndrome and primary immunoglobulin aberrations (Fudenberg 1966, Dent *et al.* 1968).

In general, the observations show that human patients with immune deficiency syndromes or on immunosuppressive treatment have a greatly increased risk of developing malignancy, chiefly of the lymphoreticular system. The interpretation is not yet certain. Conceivably a defect in the lymphoreticular system, either naturally occurring or following the use of drugs, could result in immune deficiency on the one hand and malignancy on the other. Alternatively, reduction in the efficiency of the immunological surveillance mechanism could result in failure to prevent the emergence of antigenic tumour cells from clones pre-existing in the body. It is even conceivable that multiplication of an oncogenic virus occurs unchecked in immunosuppressed patients, and that the malignancy is secondary.

Irrespective of the underlying mechanism, it is already clear that continued immunosuppression increases the risk of infections and tumours. The more effective the immunosuppression, the greater the risk. It follows that efforts should be directed towards alternative methods of ensuring homograft survival without the use of prolonged, generalized immunosuppression. Possible approaches include low dose tolerance (Dresser & Mitchison 1968) and enhancement of homografts (French & Batchelor 1969). Another possibility is the use of interferon inducers or other antiviral compounds, such as double-stranded polyribonucleotides or other polyanions which can afford protection against virus-induced leukæmias and sarcomas in rodents (Merigan & Regelson 1967, Sarma *et al.* 1969, Chirigos *et al.* 1969, Wheelock *et al.* 1969). However, too little is known about the long-term effects of these compounds in man to assess the feasibility of their use in transplant patients. At present the most hopeful long-term approach seems to be ensuring the best possible match by histocompatibility typing combined with a procedure producing tolerance or enhancement.

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The following paper was also read:

Modes of Escape from Cytotoxic Treatment

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