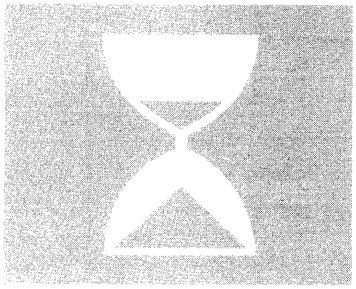
Reviews in Medical Virology *Rev. Med. Virol.* **9**: 3–14 (1999)

CLASSIC PAPER



Infectious Pulmonary Disease in Patients Receiving Immunosuppressive Therapy for Organ Transplantation†

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INTRODUCTION

The article by Hill, Rowlands and Rifkind[1] was a by-product of an institutional commitment to transplantation in 1961–63, at the University of Colorado (Denver). The programme was launched after the ostensible fantasy of exploiting transplant procedures clinically had been demonstrated to be occasionally feasible at the Peter Bent Brigham Hospital in Boston (Joseph Murray and John Merrill),[2,3] and at the Paris centres directed by Jean Hamburger[4,5] and Rene Kuss.[6] Between January 1959, and the summer of 1962, seven examples had been produced (two in Boston, five in Paris) of patient survival exceeding 6 months following human kidney transplantation, all but one after recipient cytoablation with total body irradiation (Table 1).

The seventh recipient, treated in Boston by Murray, was unique in that the patient was immunosuppressed from the outset with daily doses of the 6-mercaptopurine derivative, azathioprine.[7] This therapeutic option, which evolved from the basic investigations in rodents by Schwartz and Dameshek,[8,9] and of Meeker and Good *et al.*,[10] had been greeted initially with feverish enthusiasm, in part because it had been recognized that the strategy of cytoablation with total body irradiation would permit success in only occasional cases of human renal transplantation. The only evidence that this could be achieved in animals was the survival for 73 days of a beagle dog before dying of pneumonia reported by Mannick *et al.*[11] However, this animal also had been given donor bone marrow and was suspected of having received the allograft from a closely related donor.[12]

Abbreviation used: GVHD, graft versus host disease.

Accepted 21 September 1998.

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CCC 1052–9276/99/010003–12 $17.50

The optimism generated by the new drugs was quickly dampened by the results of their preclinical studies by Calne[13,14] and Zukoski[15] with the outbred mongrel canine kidney transplant model in which only about 5% of the dogs survived for ≥100 days.[16] In Murray's clinical trials,[16,17] survival did not appear at first to be substantially better than with irradiation. Patient 7 in Table 1 was the only one of the first ten in Murray's clinical trial (two using 6-MP, eight with azathioprine) who had ≥6 months graft function. An overwhelming rate of patient death usually was caused by, or related to, infection.

The dilemma, as it was perceived at the time, is shown in Figure 1. It was feared that chronic drug immunosuppression powerful enough to prevent organ allograft rejection would render the recipient hopelessly vulnerable to indigenous and environmental pathogens. It also was suspected that immune surveillance to tumours would be eroded, a possibility that ultimately was verified.[18] At the nadir of the consequent pessimism in 1962–63, two reproducible observations were made at the University of Colorado (Denver) that shaped future developments in organ transplantation.[19]

First in dogs and then in humans, kidney allograft rejection, which had been considered to be one of nature's strongest reactions, was shown to be readily reversible by adding high doses of prednisone to baseline azathioprine therapy. More importantly, the allografts appeared to self induce tolerance.[19] This was manifested by the ability in the Colorado live donor recipients to subsequently wean maintenance immunosuppression to doses below those which had failed at the outset to prevent rejection. Exploitation of these findings resulted in the first series of successful renal transplantations. Nine of the first ten patients had prolonged survival[19] and four lived for >25 years.[20] Two of the four bear the longest surviving organ allografts in the world today (>35 years). These two patients, whose transplant operations were separated by a 9 day interval, have been without immunosuppressive drugs for 4 and 32 years, respectively.

Table 1. Kidney transplantation ≥6 months survival as of March 1963

Case	City[Ref]	Date	Donor	Survival (months)[a]
1.	Boston[2,3]	24-1-59	Frat twin	>50
2.	Paris[4]	29-6-59	Frat twin	>45
3.	Paris[6]	22-6-60	Unrelated[b]	18 (Died)
4.	Paris[5]	19-12-60	Mother[b]	12 (Died)
5.	Paris[6]	12-3-61	Unrelated[b]	18 (Died)
6.	Paris[5]	12-2-62	Cousin[b]	>13
7.	Boston[7]	5-4-62	Unrelated	10

[a]The kidneys in patients 1, 2, and 6 functioned for 20.5, 25, and 15 years respectively. Patient 7 rejected his graft after 17 months and died after return to dialysis.
Boston J. E. Murray (cases 1 and 7)
Paris: J. Hamburger (cases 2, 4 and 6)
R. Kuss (cases 3 and 5)
[b]Adjunct steroid therapy.
All patients received cytoablation with total body irradiation, except number 7 who was given azathioprine.

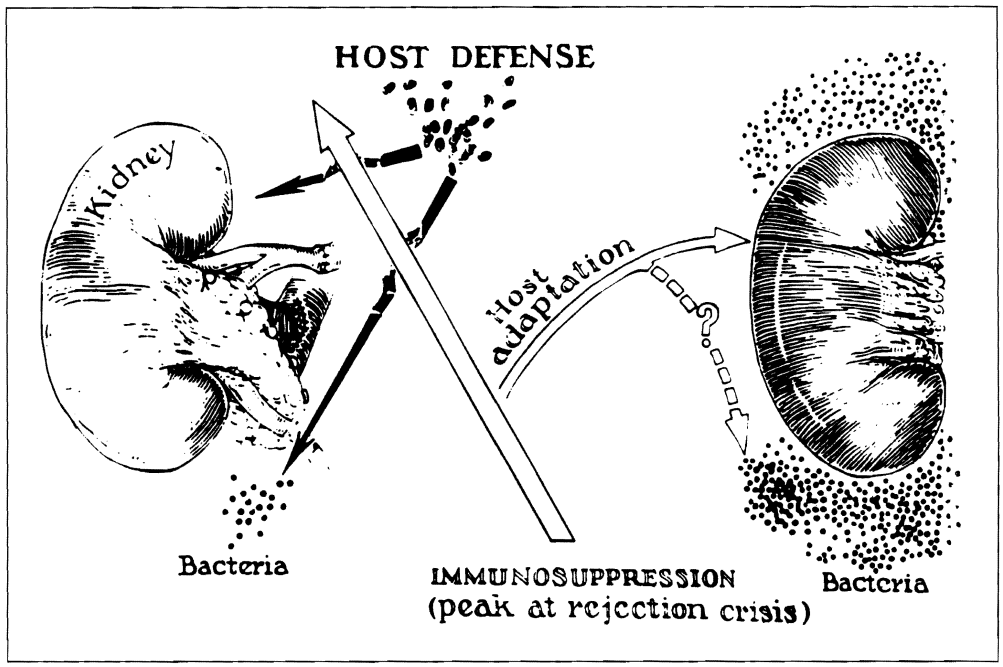

Figure 1. The original caption for this figure: 'Possible mechanisms of simultaneous loss of host reactivity to specific strains of endogenous bacteria, as well as to the alien renal tissue.' (By permission of *Surgery* **56**, 296, 1964).

The early kidney experience triggered the first attempts in Colorado in 1963 to replace the liver,[21] a clinical trial with baboon renal xenografts,[22] and transplantation of the spleen for anti-tumour objectives,[18] All of these cases were failures, as well as three others in which irreparably damaged cadaver kidneys were transplanted.[23] accounting for almost half of the autopsies in the Hill–Rowlands–Rifkind report. In contrast, when the high rate of success of the double drug immune suppression (i.e. azathioprine/prednisone) in the live-donor kidney cases became known, a world wide proliferation of renal transplant centres occurred within the succeeding 12 months.

Nevertheless the sober report of Hill, Rowlands and Rifkind gave warning that the concept depicted in Figure 1 was correct, at least in part. In this autopsy collection, infections for which specific antibiotics were available had been largely controlled, while opportunistic microorganisms of normally low pathogenicity were over-represented. Of these, CMV was the most common

and lethal, presaging a problem that remains endemic today. Although *Pneumocystis carinii* was found at only one autopsy, its presence as a co-infection with CMV was soon to be reported separately by one of the authors (Rifkind *et al.*[24]), anticipating this combination of infectious agents in the AIDS epidemic that lay ahead.

INFECTIOUS PULMONARY DISEASE IN PATIENTS RECEIVING IMMUNOSUPPRESSIVE THERAPY FOR ORGAN TRANSPLANTATION*

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Reprinted from *N. Engl. J. Med.* **271,** 1021–1027 (1964)

Whole-organ homotransplantation is a modality of therapy that has only recently been submitted to clinical investigation in man,[1] and reports on the natural history of patients so treated are therefore fragmentary. The clinical course of such patients obviously is profoundly influenced by the chemotherapy that they receive, usually including corticosteroids and immunosuppressive antimetabolites. These compounds have a wide range of effects on the host metabolic reactions, and they so alter immunologic responsiveness that changes in the spectrum of infectious disease may reasonably be expected.

Up to February, 1964, 61 patients had received homotransplants or heterotransplants at this center; of these 32 had died after periods ranging from one to two hundred and seven days. In 26 of the 32 fatal cases active pulmonary infection was demonstrable at autopsy, and in 3 others, without pulmonary disease, serious infections of other organs were present. This study is concerned with the pulmonary infections, and reports a high incidence of infection with organisms not commonly associated with systemic disease.

CLINICAL AND PATHOLOGICAL METHODS

The patients were selected for transplantation according to criteria published elsewhere.[2] Renal homotransplants were performed with the use of various donors — living relatives, living nonrelatives and cadavers; renal heterotransplants were from papio species (baboon). Hepatic homotransplants were performed with the use of cadaveric donors. For splenic homotransplants, donors were preimmunized with extracts of the recipient's cancer in Freund's adjuvant, in an effort to direct the donor spleen's immunologic mechanisms against the recipient's cancer. All recipients were deemed to have progressive, incurable, fatal disease, and were carefully screened for other disease to ensure optimum potentiality for success of the graft.

Immunosuppression was achieved with azathioprine,¶ prednisone and actinomycin C|| in accordance with principles previously reported.[3,4] In general, azathioprine was used for an average of ten days before transplantation; in the postoperative period the dose was increased to the maximum tolerated (400 to 1000 mg per day), as reflected by the appearance of leukopenia. Prednisone, 150 to 400 mg daily, was given until the rejection phenomenon was suppressed, after which the dosage was decreased and in several cases discontinued. Both drugs were temporarily increased at any evidence of rejection, and in some cases 2 to 6 doses of actinomycin C, 200 μg daily, were added. Azathioprine was continued in a maintenance dose of 3 to 4 mg per kilogram of body weight per day in all patients.

At the appearance of clinical infection, therapy in the form of specific antimicrobial agents was employed in accordance with the results of bacteriologic studies.

At autopsy in most cases culture was taken for bacteria and fungi from grossly abnormal areas of the lung. In 10, fresh lung tissue was homogenized in a medium containing 10 per cent calf serum, quick frozen and stored at − 70°C. Aliquots were then inoculated into MAF and WI-26 lines of human embryonic fibroblasts in the hope of demonstrating the specific cytopathic effect of cytomegalovirus. The cultures were observed over periods of three or four weeks, and at least 1 blind passage was made before this specimen was discarded as negative.

At least 5 specimens of lung from each patient were fixed in formalin, and sections stained with hematoxylin and eosin. Periodic acid-Schiff, Brown and Brenn, methenamine silver, Ziehl-Neelsen and Giemsa stains were employed for the demonstration of microorganisms.

OBSERVATIONS

All transplant recipients who died during the period of this study were autopsied. Table 1 summarizes the facts concerning the type of transplant, therapy and infectious pulmonary disease in the 32 fatal cases. The cases are listed according to length of survival after operation. Where loss of a transplanted organ necessitated a second graft, the duration of survival was measured from the initial procedure.

Twenty-six patients, or 81 per cent of this series, died with pulmonary infection. A wide variety of agents were identified, including bacteria and fungi, as well as *Pneumocystis carinii* and cytomegalovirus. other unidentified organisms of fastidious cultural requirements and unusual staining characteristics cannot be ruled out. Many patients had multiple infections. Of the 6 patients without demonstrable pulmonary infection, 2 had septicemia (due to *Escherichia coli* and paracolon bacillus), and 1 had a wound abscess (mixed pyogens). The remaining 3 died of electrolyte imbalance, acute tubular necrosis and exsanguinating hemorrhage without demonstrable infection one, three and eleven days, respectively, after surgery. In 14 cases, or 44 per cent, the cause of death was clearly related to pulmonary infection.

The infectious agents identified in these lungs are listed in Table 2. Of the 45 agents involved, 10 were either common pyogenic bacteria or were unidentified, and 7 were pseudomonas species; the remaining 28 were distinctly uncommon pathogens: cytomegalovirus, 15 cases; fungi, 12 cases; and pneumocystis, 1 case.

Fifteen patients, or 47 per cent, were considered to have cytomegalovirus infection. This diagnosis was based on the presence of enlarged cells with the characteristic inclusions (Fig. 1): basophilic, Feulgen-negative, intranuclear rounded bodies surround by a clear halo. These bodies were found within enlarged nuclei whose chromatin was present in a thin rim. Fine, powdery basophilic material was often present also in the cytoplasm adjacent to the nucleus. In 3 cases similar inclusions were found in other organs, including the salivary glands, lymph nodes, liver, brain, pancreas and parathyroid glands. Of the 10 patients whose lungs were cultured for this virus, 4 had demonstrable inclusions; in no case could the virus be demonstrated in culture. It should be pointed out that cytomegalovirus is notoriously difficult to isolate, especially from frozen material,[5] and failure to do so

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A preliminary report of this material was presented at the April, 1964, meeting of the American Association of Pathologists and Bacteriologists.

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‡Assistant professor of medicine, University of Colorado School of Medicine.

§In the form of Imuran, Burroughs Wellcome and Company, Tuckahoe, New York.

¶In the form of Imuran, Burroughs Wellcome and Company, Tuckahoe, New York.

||In the form of Sanamycin, FBA Pharmaceuticals.

TABLE 1. *Type of Transplant, Therapy and Infectious Pulmonary Disease.*

CASE NO.	Transplant	Sex	Age	Survival after Transplant	Prednisone Therapy		Azathioprine Therapy	
					duration	dose range	duration	dose range
			yr.	days	days	mg	days	mg
1	Renal homograft, living donor	F	16	1		0	10	150–200
2	Renal homograft, cadaver donor	F	42	3	4	200–250	4	200–400
3	Hepatic homograft, cadaver donor	M	69	8	21	50–100	21	150–600
4	Renal homograft, living donor	M	50	11	5	75–100	9	375–1000
5	Splenic homograft	M	45	11	4	50–320	11	150–750
6	Hepatic homograft, cadaver donor	M	53	15	1	75	13	200–300
7	Renal homograft, living donor	M	54	15	9	180–200	26	100–400
8	Renal homograft, living donor	M	40	17	6	200–300	20	150–400
9	Renal heterograft	M	36	19	21	150–200	22	75–350
10	Hepatic homograft, cadaver donor	M	48	22	22	0–200	22	50–400
11	Renal heterograft	M	40	23	27	100–200	32	100–350
12	Hepatic homograft, cadaver donor	F	29	23	23	50–300	24	100–600
13	Renal homograft, living donor	M	35	24	21	80–425	26	150–400
14	Renal homograft, cadaver donor	M	21	25	12	20–150	23	100–1000
15	Renal homograft, living donor	M	49	25	11	50–200	29	200–400
16	Renal heterograft	M	45	36	2	150	43	100–250
17	Renal homograft, living donor	F	42	37	32	30–400	35	150–500
18	Renal homograft, living donor	M	25	37	37	60–200	30	100–400
19	Renal homograft, cadaver donor	M	29	40	6	30–45	31	100–900
20	Splenic homograft	M	53	41	38	30–200	41	150–400
21	Renal homograft, living donor	M	21	41	30	100–240	45	100–500
22	Renal homograft, living donor	F	25	43	43	100–200	62	100–300
23	Renal homograft, living donor	M	20	48	45	60–200	52	150–400
24	Renal homograft, living donor	M	39	48	45	5–230	41	125–440
25	Renal homograft, living donor	M	20	62	55	15–200	63	250–750
26	Renal homograft, living donor	M	41	76	76	75–200	103	100–400
27	Renal homograft, living donor	M	25	79	79	5–400	80	100–600
28	Renal homograft, living donor	F	44	83	77	5–200	88	150–400
29	Renal homograft, living donor	M	17	95	88	20–400	103	100–350
30	Renal homograft, living donor	M	34	113	99	10–100	120	100–900
31	Splenic homograft	M	47	142	142	5–30	146	100–250
32	Renal homograft, living donor	M	30	207	201	10–315	213	100–400

by no means invalidates the diagnosis. The frequency of infection with this agent was greater in the patients with the longest survival (Table 1): 7 of the 8 who survived for more than sixty days were infected. The incidence of infection had no relation to the age of the patient (Table 3).

An unusual spectrum of histologic reactions to the infectious organisms was present. This was presumably due to alterations in cellular immune mechanisms and bone-marrow suppression brought about by transplantation and immunosuppression. In a few cases a classic bronchopneumonia was seen, with diffuse or peribronchial congestion, edema and polymorphonuclear infiltration. A characteristic pattern, however, most commonly associated with candida and pseudomonas infection, was the appearance of widespread but sharply limited lesions (Fig. 2). In these, a small number of alveoli were filled with blood and proteinaceous fluid, usually in the vicinity of a small arteriole whose wall was massively invaded with bacilli or fungi. These lesions were remarkable for the absence of polymorphonuclear leukocytic reaction. In 1 case lesions of this type were so closely associated with the presence of inclusions that an etiologic relation is suggested, but in others, no-tissue reaction was evident.

Poor or absent leukocyte response was a common feature. In 1 case, for instance (Case 26), abscesses containing amorphous debris and tiny gram-negative bacilli were in the midst of regions of alveoli packed with macrophages containing large numbers of similar organisms. No polymorphonuclear leukocytes were present (Fig. 3). The organism did not grow in culture. In another patient (Case 19) staphylococcal abscesses, which are classically associated with a brisk polymorphonuclear response, were marked by peripheral granulation tissue and a mild to moderate lymphocytic reaction.

A particularly violent necrotizing effect of fungal organisms was noted. In all 10 cases of candidiasis, as well as in those of aspergillosis and nocardiosis, fungal hyphae and pseudohyphae were associated with necrosis characterized by abundant "nuclear dust," abscesses, thick, proteinaceous exudate and variable but usually minimal polymorphonuclear reaction (Fig. 4).

Mixed infections were common. In only 4 cases was cytomegalovirus unaccompanied by another infectious agent, such as pseudomonas, candida, pneumocystis (Fig. 5) or nocardia. In at least 4 cases 3 microbial agents were present concurrently (Table 1).

TABLE 2. *Infectious Agents Encountered.*

AGENT	No. of Infections
Cytomegalovirus	15
Candida sp.	10
Aspergillus	1
Nocardia	1
Pneumocystis	1
Pseudomonas sp.	7
Miscellaneous bacteria	10
Total	45

TABLE 1. *(Concluded)*

CASE NO.	No. of Doses of Actinomycin C (200 Microgm)	Clinical Evidence of Pneumonia	Pneumonitis at Autopsy	Responsible Organisms	Inclusions	Cause of Death
1	0	0	0			Electrolyte imbalance
2	0	0	Minimal	?		Uremia
3	0	0	+	Cytomegalovirus; candida sp.	+	Pulmonary infarct
4	0	+	+	Cytomegalovirus; *Pseudomonas aeruginosa.*	+	Sepsis (pseudomonas)
5	3	0	0			Rupture of spleen; carcinoma of stomach, metastatic.
6	0	0	+	*Mycobacterium tuberculosis*		Pulmonary emboli
7	5	+	+	Pseudomonas sp.		Pulmonary infarct
8	7	0	0			Sepsis (paracolon bacillus)
9	4	0	+	Klebsiella		Uremia
10	9	0	+	Gram-negative rods; candida sp.		Pulmonary infarct
11	4	+	+	Candida sp.; *Ps. aeruginosa.*		Pulmonary infarct
12	4	0	0			Peritonitis *(Escherichia coli)*
13	11	0	0			Congestive heart failure
14	6	0	0	Cytomegalovirus	+	Sepsis (pseudomonas)
15	2	+	+	Cytomegalovirus; *Esch. Coli; M. tuberculosis.*	+	Sepsis *(Esch. coli);* pulmonary emboli.
16	9	+	+	Cytomegalovirus; *Ps. aeruginosa.*	+ + +	Uremia; pulmonary emboli.
17	31	0	+	?		Septic pulmonary infarct
18	6	+	+	*Aspergillus fumigatus; Candida albicans.*		Sepsis (aspergillus & candida)
19	2	+	+	*Staphylococcus aureus*		Exsanguination
20	0	+	+	Cytomegalovirus; *Candida stellatoidea.*	+ + +	Pneumonia
21	2	0	+	Cytomegalovirus; candida sp.	+	Pyelonephritis; myocarditis.
22	8	0	+	*Diplococcus pneumoniae*		Uremia; peritonitis *(D. pneumoniae).*
23	6	0	0	Cytomegalovirus	+ +	Congestive heart failure
24	2	+	+	*Ps. aeruginosa*		Sepsis (Pseudomonas)
25	15	0	0	Cytomegalovirus	+ + + +	None known
26	15	+	+	Cytomegalovirus; candida sp.; gram-negative intracellular rods.	+ + +	Sepsis (candida)
27	22	+	+	Cytomegalovirus; candida sp.; *Ps. aeruginosa.*	+ + +	Sepsis (pseudomonas & candida)
28	27	0	0	Cytomegalovirus	+ + + +	Pancreatitis
29	20	+	+	Cytomegalovirus; *Pneumocystis carinii.*	+ +	Pneumonia (pneumocystis)
30	11	+	+	Candidi sp.; *Ps. aeruginosa.*		Sepsis (candida & pseudomonas)
31	0	0	+	Cytomegalovirus; gram-positive cocci.	+ + +	Pneumonia; carcinoma of stomach, metastatic.
32	38	+	+	Cytomegalovirus; *C. albicans; Nocardia asteroides.*	+	Generalized nocardiosis

Clinical evidence of biologic response to the infecting organisms was lacking in a high percentage of patients. Fifty per cent of those who had pulmonary infection at autopsy had not given evidence of this infection clinically. Four were due to cytomegalovirus, 1 to *Mycobacterium tuberculosis*, 3 to candida species, and the remaining 5 to various bacteria. In some cases there was lack of tissue response to the infecting organism, and in others lack of general response in terms of fever, leukocytosis and so forth.

TABLE 3. *Distribution of Cytomegalovirus Infection According to Age.*

AGE GROUP *yr.*	No. of Patients	No. with Inclusions	Percentage with inclusions
16–29	11	6	54
30–39	5	1	20
40–49	11	5	45
50–70	5	3	60

No clear relation between daily drug dosage and severity or type of infection emerges from this study. On the other hand, it is evident that survival and cumulative drug dosage correlate with the incidence and type. All the patients dying without demonstrable infection lived for less than twenty-four days after transplant. All the patients with triple infections, on the other hand, lived for more than twenty-four days. The incidence of fungous and cytomegalovirus infections increased with length of survival.

DISCUSSION

The transplant recipient has joined the growing legion of patients whose therapist treads a think, barely observable line: too little

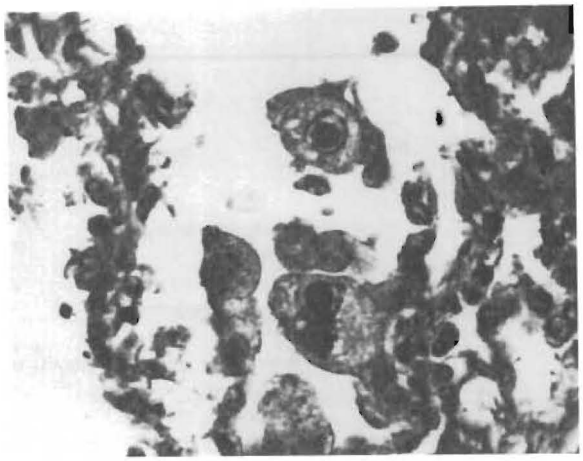

FIGURE 1. *Photomicrograph of the Lung in Case 27 (Azan Stain X650).*

Two cytomegalic cells, one with two affected nuclei, are present. Characteristic are the intranuclear inclusions, peripheral rim of nuclear chromatin and large mass of cytoplasm. In many cases, the cytoplasm also contains small, granular basophilic inclusions.

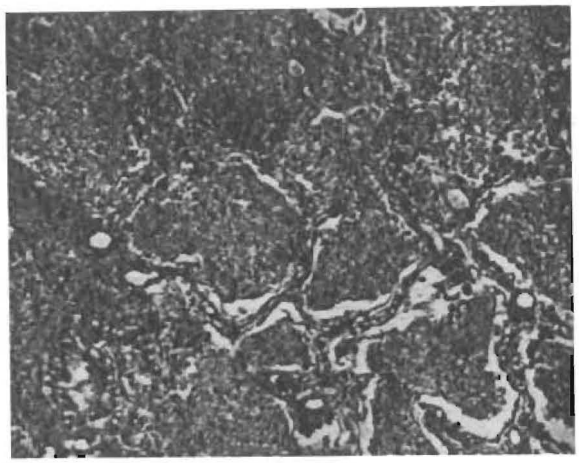

FIGURE 3. *Field Adjacent to an Abscess in Case 26 (Hematoxylin and Eosin Stain X100).*

The alveoli are filled with macrophages whose voluminous cytoplasm can be demonstrated with special stains to contain large numbers of small gram-negative rods. No acute inflammatory reaction is present.

therapy, and the patient succumbs to his biologic abnormality; and too much, and a new constellation of unrelated but equally ominous pathologic processes arises. To find and stay on the narrow middle road requires alert individualization of dose and a considerable delicacy of response to changes in the patient's condition. Of the 32 transplant recipients in this series, death in 26 was related to complications of therapy, usually infection.

The 26 patients who had pulmonary infection at death are the subject of this report. All patients received azathioprine and prednisone, and some actinomycin C (Table 1), and the administration of each of these drugs is known to be associated with an increase in incidence of infection. The anti-inflammatory action of cortisone,[6] the inhibition of RNA synthesis by actinomycin[7] and the "immunosuppressive" and bone-marrow-depressant actions of azathioprine[8,9] are usually invoked, but additional mechanisms deserve consideration.

The anti-inflammatory effect of cortisone and related compounds has been known for a long time, but a firm biochemical basis for this has not been established. Among the biologic effects of adrenocortical hormones are the inhibition of fibroblast replication and collagen production,[10] inhibition of chemotaxis,[11] lysis of lymphoid tissues[12] and suppression of the formation and activity of interferon.[13] An interesting recent observation is that of Weissmann and Thomas,[14] who have demonstrated stabilization of the lysosomal membrane by certain corticoids. The lysosome, an intracellular membrane-enclosed sac of lytic enzymes, is conceived of by deDuve,[15] Novikoff,[16] and Hirsch[17] and others as instrumental in the intracellular digestion of phagocytized particles; if lysosomes were so stabilized by corticoids that their complement of enzymes could not reach and digest phagocytized parasites, these parasites would presumably multiply and kill the host cell. That cortisone may indeed act in this way is given support by the experiments of Iwata et al.,[18] who demonstrated germination of phagocytized aspergillus spores intracellularly in isolated peritoneal leukocytes from cortisone-treated animals, but not in cells from control animals.

Some controversy exists regarding the effect of the actinomycins on immune responses[19]; their demonstrated primary action is complete inhibition of DNA-dependent RNA synthesis, and thus secondarily inhibition of protein synthesis, presumably through lack of appropriate messenger RNA.[7] The effect in both in vitro and in vivo systems is immediate and lasts no more than two days, and in the experiments in which an effect on immune-globulin production has been demonstrated, the effect was a delay in antibody synthesis, not total inhibition.[19] Since doses of actinomycin C in the patients in this

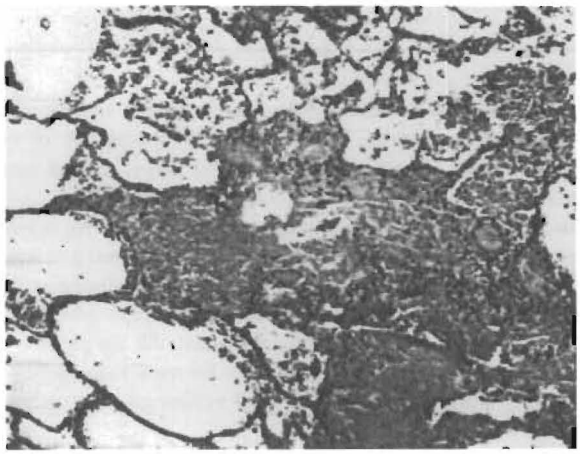

FIGURE 2. *Sharply Delimited Region of Alveolar Hemorrhage and Edema in Case 21 (Hematoxylin and Eosin Stain X65).*

This patient had candida and cytomegalovirus infections, but inclusions were not clearly related to the foci of the type illustrated.

FIGURE 4. *Section in Case 32, Showing an Abscess, in Which Nocardia Hyphae Are Numerous, That Is Surrounded by a Cloud of Degenerating Cells, Nuclear Debris and Matted Hyphae (Hematoxylin and Eosin Stain X35 — Insert: Brown–Brenn Stain of Fungal Hyphae X675).*

The adjacent alveolar septums are thickened, and macrophages are present in the alveoli.

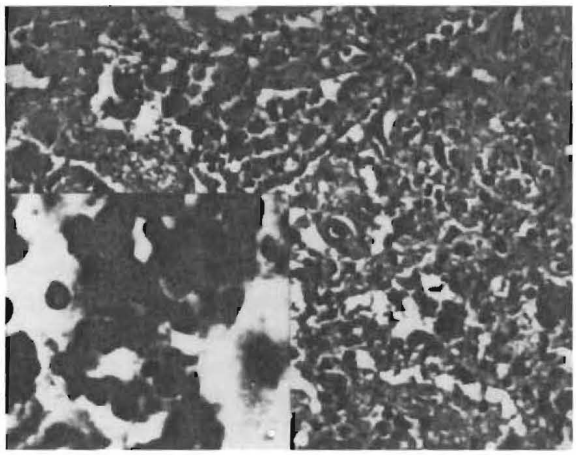

FIGURE 5. *Section in Case 29, Showing Alveoli Containing Bubbly, Proteinaceous Material That Is PAS-Positive and within Which Cysts of Pneumocystis carinii Are Readily Demonstrable in Large Numbers with Appropriate Stains (Hematoxylin and Eosin Stain X250 — Insert: Pneumocystis Cysts, Grocott Methenamine Silver Stain X575).*

Hyperplasia of the septal lining cells is clearly seen, and a cytomegalic cell is near the center.

series were infrequent and widely spaced they presumably acted in acute situations such as imminent rejection crises,[20] rather than on immune mechanisms related to smoldering infections.

Azathioprine, an imidazole derivative of 6-mercaptopurine, has had widespread use as an immunosuppressive drug in the past few years. Its effect appears to be similar to that of 6-mercaptopurine,[9] but available data suggest that it may have a higher therapeutic index in suppression of the renal-homograft response.[21] Bone-marrow suppression, entirely similar to that seen in 6-mercaptopurine therapy, occurs regularly with higher doses. An increased incidence of infection is therefore not unexpected. Although suppression of immune response to infectious agents by this drugs has not been demonstrated, depression of antibody response to other antigenic proteins has been reported.[8]

The specific drug effects that are directly responsible for such an unusual spectrum of diseases are unknown, as are the possible profound effects of the continued interaction of foreign protein (in the form of the transplanted kidney) and the host. The predominance of fungous and viral infectious in particular is intriguing. A recent editorial by Beeson[22] calls attention to the unexplained association of specific infectious agents with specific noninfectious diseases, citing mucormycosis and diabetes, salmonellosis and sickle-cell disease, disseminated herpes zoster and chronic lymphocytic leukemia, pneumococcal peritonitis and the nephrotic syndrome, candidiasis and hypoparathyroidism.[22] Attention has recently been called to the association of nocardiosis and pulmonary alveolar proteinosis.[2] Cortisone treatment of rabbits and rats has frequently been associated with pneumocystis pneumonia,[24,25] and the intriguing observation has recently been made that pneumocystis pneumonia in children may occur during tapering of prednisone therapy as the dose drops below 1 mg per kilogram of body weight per day.[26] A similar association of fungous infections with prolonged antimetabolite and cortisone therapy is suggested in the present report. The studies of Sidransky et al.[27,28] showing enhanced germination and poor host response to aspergillus in cortisone-treated and antimetabolite-treated mice may be further evidence for this view.

The part played by bone-marrow depression in these infections is difficult to assess. Bone-marrow aspirations and biopsies were not performed regularly during life, and the state of the bone marrow at death obviously bore no necessary relation to its state during the postoperative course of the patient. Hypoplasia of the bone marrow, present in many of these cases at death, could not be correlated with severity or type of infection, and a normal bone marrow was present in some cases notable for the absence of leukocytic response to the infecting organism. It seems reasonable to hypothesize that infections arising during periods of bone-marrow depression could not be reversed by re-establishment of a normal marrow.

Clinical disease caused by cytomegalovirus has been recognized largely in premature or debilitated infants. The wide asymptomatic distribution of the virus is attested to by its presence in the salivary glands of 10 per cent of infants at autopsy, and by the demonstration of complement-fixing antibodies to the agent in about 80 per cent of adults past the age of thirty-five years.[5,29] The data presented here, with 15 of 32 autopsied patients showing infection, support the concept of a wide distribution of the virus among the population.

Page, Condie and Good[30] have recently suggested that 6-mercaptopurine is virucidal in patients with chronic hepatitis, but an inhibitory effect of a closely related drug, azathioprine, on virus replication is not suggested by this study. The high incidence of demonstrated cytomegalovirus infection has been mentioned. Study of the brains of these autopsied patients suggests the presence of viral infections in a very high incidence.[31] Prolonged survival and pathogenicity of hepatitis virus is inferred in a kidney-transplant recipient, not included in this report, who has had smoldering acute hepatitis for six months while under continuous prednisone and azathioprine therapy. Pathological examination of a recent liver biopsy reveals continuing active acute hepatitis, without cirrhosis or the usual features of chronic hepatitis.[32]

SUMMARY

Pulmonary infections were present terminally in 26 of 32 patients dying one to two hundred and seven days after receiving an organ transplant. The responsible organisms included a large number of unusual agents not commonly producing clinical disease. Candida, aspergillus, nocardia, pneumocystis and cytomegalovirus were identified in 28, and various bacteria, notably pseudomonas, in 17 cases. Multiple infections were common. The metabolic effects of the immunosuppressive drugs and corticosteroids received by these patients are discussed in relation to the high incidence of unusual infections.

Since this report was submitted for publication 7 more patients have died after survivals of thirty-seven to one hundred days. All 7 had pulmonary infections, including 5 with cytomegalovirus, 2 with **aspergillus**, 2 with pneumocystis, 2 with candida, 2 with pseudomonas, 2 with ***Staph. aureus*** and 1 with tiny gram-negative intracellular bacilli similar to those seen in Case 26. Recently, one of us (D.R.) isolated a cytopathogenic agent, apparently cytomegalovirus, from the lungs of 2 patients in whom inclusion bodies had been demonstrated histologically at autopsy.

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COMMENTARY

Early workers in transplantation[25,26] including Hill, Rowlands and Rifkind recognised the resemblance of allograft rejection to the response against infections associated with delayed hypersensitivity, as exemplified by tuberculosis. With the demonstration of the MHC restricted mechanisms of adaptive infectious immunity by Doherty and Zinkernagel in 1973[27–30] it seemed obvious that allograft rejection must be the physiological equivalent of the response to this kind of infection.

Microorganisms that generate such an adaptive immune response are generally intracellular, and have no or low cytopathic qualities.[31] Although MHC-restricted host cytolytic T lymphocytes recognise only infected cells, elimination of all the infected cells could disable or even kill the host. Consequently, mechanisms have evolved that can temper or terminate the immune response, allowing both host and pathogen to survive.[31,32] They are the same mechanisms that allow survival of allografts.[33]

The highly variable clinical manifestations of these mechanisms can be illustrated by the different outcomes that may follow an infection with disseminated non-cytopathic microorganisms (e.g. CMV and other pathogens identified by Hill, Rowlands and Rifkind, or the common hepatitis viruses). These are shown in Figure 2, with transplantation analogies shown below each panel. After a rapid increase during the so-called latent period of a non-cytopathic infection, the pathogen load may be dramatically and efficiently reduced by antigen-specific effector T-cells, following which the CTL subside (Figure 2, left panel). The events are analogous to those of rejection in an untreated or inadequately immunosuppressed transplant recipient. Alternatively, such infections may lead to a continuously high antigen load and an antigen specific immunologic collapse (Figure 2, second panel), equivalent to allograft acceptance (and tolerance). Persistence of the infectious agent with an unrelenting immune response (Figure 3, third panel) may result in serious immunopathology (e.g. chronic active hepatitis with HBV or HCV infection), analogous to either chronic rejection or uncommonly graft versus host disease (GVHD).

Although the relation between allograft rejection and the adaptive immune reaction against non-cytopathic microorganisms was unmasked by the discoveries of MHC restriction, the means by which antigen specific non-reactivity (tolerance) is induced has remained one of the most controversial questions in biology. Zinkernagel has approached the enigma with experimental models that place viral and transplantation immunity on common ground.[31,32,34]

Using transgenic techniques, colonies of donor animals are produced whose transgenes express viral epitopes,

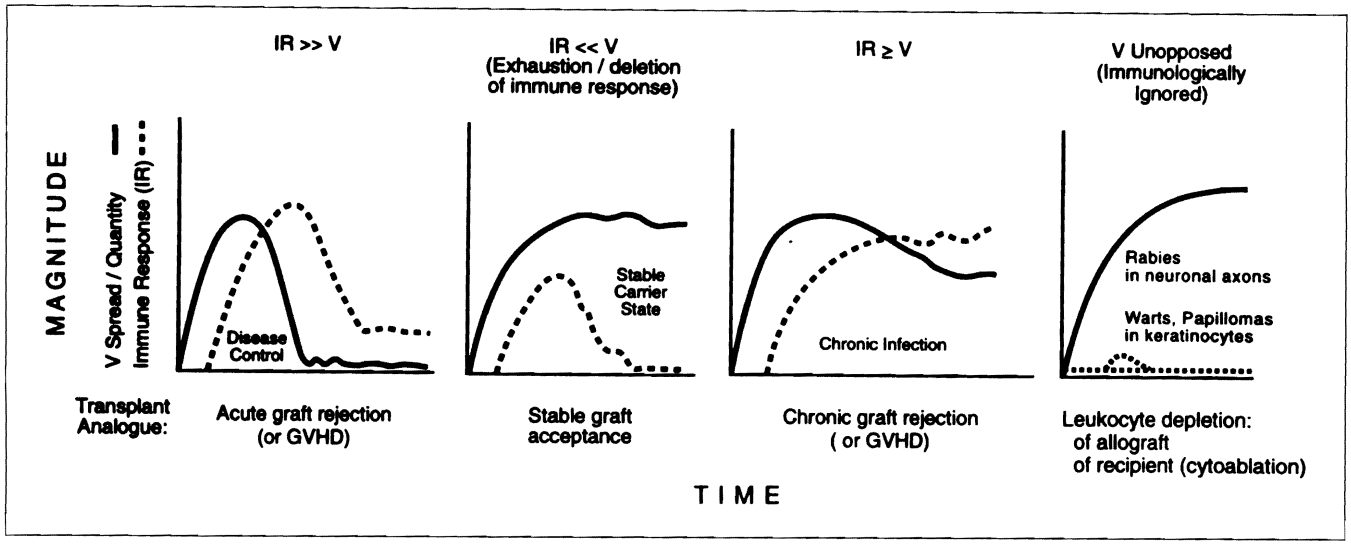

Figure 2. Variable outcomes after infection with widely disseminated non-cytopathic viruses (or other microorganisms) and analogies (given below each panel) to organ and bone marrow transplantation. The horizontal axis denotes time, and the vertical axis shows the viral load (v, solid line), and the host immune response (IR, dashed line).

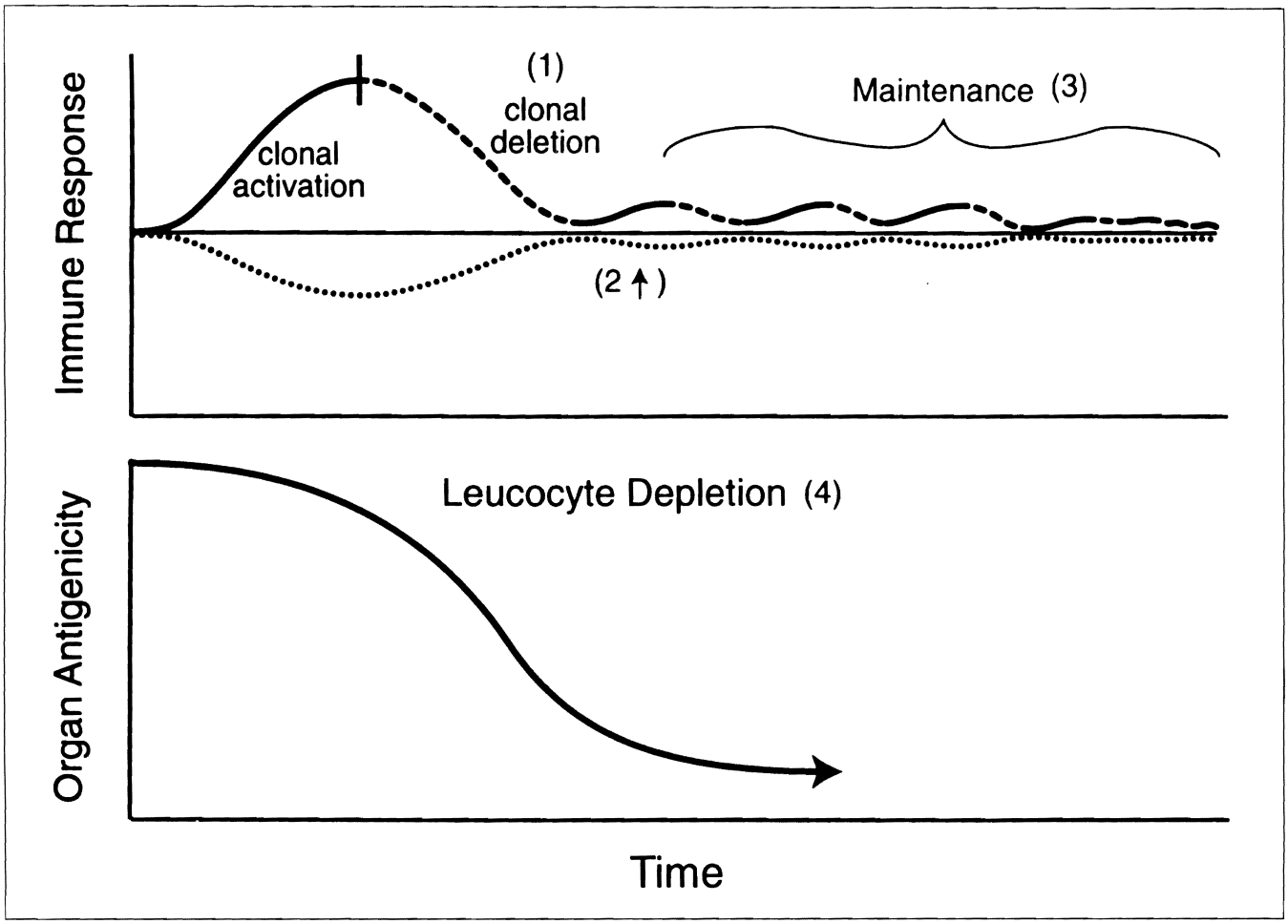

Figure 3. The four events that occur in close temporal approximation when there is successful organ engraftment: above, double acute clonal exhaustion (1,2) and subsequent maintenance clonal exhaustion (3) plus, below, loss of organ immunogenicity due to depletion of the graft's passenger leukocytes (4). (By permission of *Nature Med.* **4,** 1006–1007, 1998).

but are otherwise syngeneic with recipients, in effect creating a highly defined minor histocompatibility barrier (e.g. the gp33 peptide of LCMV). Alternatively, recipient effectors that play a known role in responding to specific viral epitopes such as gp33 can be modified or replaced.[34] With these technologies, it has been possible to pool information in viral immunology (particularly that acquired about LCMV) with that of transplantation

Table 2. Central therapeutic dogma of immunosuppression

Strategy	Baseline agents	Sites of inhibition
1. Baseline therapy with one or two drugs	Azathioprine[a]	DNA synthesis
2. Secondary adjustments with steroids or antilymphoid agents	Cyclosporine	Interleukin-2 production
3. Case to case trial (and potential error) of weaning.	Tacrolimus	Interleukin-2 production

[a]Equivalent results with cyclophosphamide.

immunology and penetrate the mystique of tolerance.[35]

Under circumstances of either a non-cytopathic infection or transplantation, antigen specific non-reactivity requires only two mechanisms, both of which are controlled by antigen migration and localisation.[33] A prerequisite for the first of these, clonal exhaustion/deletion, is antigen presentation to organised lymphoid collections in lymphoid organs (i.e. spleen, lymph nodes, bone marrow, thymus) or in ectopic locations (e.g. the graft itself). It was recognised in 1992 that this prerequisite was being met after organ transplantation by the lymphoid oriented migration of passenger leukocytes from transplanted organs. The result was widespread immune activation of the recipient, leading to clonal exhaustion.[33,36,37] More importantly, survival of the donor derived leukocytes for up to 30 years in 30 of 30 recipients with continuously functioning organ allografts implied an ongoing role for these peripheralised cells[33,35–38].

The second mechanism of non-reactivity is avoidance by the antigen of lymphoid collections, thereby precluding rather than causing efficient immune activation. This strategy is exemplified in infectious disease by the migration routes of rabies and wart viruses, and in experimental transplantation models by pre-transplant depletion of passenger leukocytes from the allograft[33,36,37] (Figure 2, right panel).

The two mechanisms can be readily identified in most infections, but their study is complicated in most transplantation circumstances by the presence of two populations of immune competent cells (i.e. donor as well as recipient), each of which can induce tolerance to the other.[33,36–38] With the chimerism discoveries in 1992, it was possible to explain the results accomplished with the combined use of azathioprine and prednisone in 1962–63, and why in later years, the treatment algorithm developed with these drugs (Table 2) readily accommodated the replacement of azathioprine by more potent T-cell directed baseline drugs (i.e. cyclosporine and tacrolimus). The same applied to a succession of adjunct agents: antilymphocyte globulin and other antilymphoid preparations, mycophenolate mofetil and other 'second line' drugs.

The results with kidney transplantation improved with the more potent drugs, and it became possible to effectively transplant a full spectrum of extrarenal organs. However, the characteristic cycle of immune activation and various degrees of subsequent immune exhaustion, aided by an element of secondary immune indifference, has remained the same. For organ engraftment to occur, four interrelated events, all regulated by antigen migration and localisation[33] must occur in relatively close temporal proximity[35–38] (Figure 3): (1) and (2), reciprocal antigen specific clonal exhaustion/deletion of the host versus graft and graft versus host immune reactions, (3) maintenance exhaustion, and (4) reduction of the leukocyte-depleted allograft antigenicity, making the transplanted organ an object of 'immune indifference' to the host immune system.

Because immunity and tolerance to alloantigens follow the same rules as the response to microbial antigens,[33] it is not possible with current transplantation practices to induce tolerance to allografts on one hand without risking unwanted tolerance to pathogens on the other. Furthermore, conventional bone marrow transplantation is only a mirror image of the events after organ transplantation[33,36–38] with the same governance of the immune events by antigen migration and localisation.[33] Although pretransplant cytoablation renders the recipient subject to GVHD, the host leukocytes are not all eliminated.[39] The weak host versus graft reaction mounted by the remaining recipient cells, and the parallel graft versus host reaction of the donor cells can eventually result in reciprocal tolerance, all the while with the threat of undesirable induced non-reactivity to pathogens.

Given the historical perspective of this classic paper, what does the future hold? If strategies to develop xenotransplantation are to be developed, it must be recognised that the barrier to xenografts is analogous to the immune defense against *cytopathic* microorganisms of the kind that were underrepresented in the cases reported by Hill, Rowlands and Rifkind.[1] These pathogens are typically extracellular and activate the full resources of the innate as well as the adaptive immune system.[31] Because there is no MHC-restricted safety valve, an uncontrollable innate immune response is provoked by discordant xenografts expressing the gal-alpha-1,3-gal carbohydrate linkages, an epitope which also is found on numerous cytopathic bacteria, protozoa and viruses.[40]

The clinical use of such discordant animal donors will require changing the xenogeneic epitope to one that

mimics a non-cytopathic profile.[41] Blocking activation of complement (reviewed in[42]) will not suffice. The alternative is elimination of the xenogeneic epitope, an objective currently beyond reach in the pig (the most widely advocated donor animal) because of the unavailability of stem cells for gene deletion in this species. However, if these innate responses can be avoided, then we can anticipate problems from non-cytopathic viruses such as those described in this classic paper.

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