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The local graft versus host reaction in the rat as a tool for drug mechanism studies

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Summary

1. A local graft versus host reaction in rats permits the evaluation of drug action on (i) donor (parental) animals and (ii) recipient animals (F_1 hybrid) prior to establishing a lymphoid cell graft to detect drugs which might attenuate immunocompetence over a prolonged period (e.g. steroids).

2. Treatment of the recipient animals after a lymphoid graft readily detects anti-proliferative agents and may disclose other agents which interfere with the recognition of non-self by viable graft cells.

3. Treatment of the cell graft *in vitro* before innoculation into the recipient animal permits the detection of alkylating agents, wihch effectively deactivate graft cells at non-toxic concentrations.

4. Examples are given of how this reaction may be used to evaluate functionally the effects of chemical modification of the cell surfaces of lymphocytes and to detect active metabolites generated from a precursor drug (e.g. cyclophosphamide).

5. Some novel immunosuppressant drugs are described.

Introduction

A cellular graft of lymphocytes from a parental strain of rat when injected into the paws of F_1 hybrid (i.e. tolerant) rats causes the local draining lymph nodes to become considerably enlarged, with maximal hypertrophy 7 to 9 days later. This response is called a local graft versus host reaction. When a viable parental lymphoid cell is placed within a hybrid bred from this parent, the hybrid does not recognize the parent cells as foreign and subsequently does not reject the parent cells. However, the parental lymphoid cells recognize the histoincompatible character of the other parental strain (from which the hybrid was bred) and reacts against it within the hybrid host. Thus the graft i.e. the parental cells reacts to the host (graft versus host). This reaction is manifest as 'homologous disease' when parental cells are given to animals which are immunologically incompetent (newborn, irradiated or chemically immunosuppressed). In adult animals, when parental cells are administered systemically, the graft versus host reaction is expressed by splenomegaly and/or enhanced reticuloendothial phagocytosis.

In preliminary communications (Levy, Beck & Whitehouse, 1972; Levy & Whitehouse, 1973), we drew attention to the feasibility of adapting the local graft versus host reaction in rats, originally described by Levine (1968) and others (Ford, Burr & Simonsen, 1970; Yoshida & Osmond, 1971) to the pharmacological

evaluation of potential immunosuppressant drugs. Drugs may be applied to either the parental animal donating the cells, the hybrid recipient of the graft, or to the graft cells themselves *in vitro* while in transit from the donor to the recipient.

This report describes our experience with standard immunosuppressant drugs in this assay, and its value in confirming the potential immunoregulatory activity of some novel drugs. We have also found it to be a useful means of detecting irreversible lymphocyte-deactivating activity among putative metabolites or potential drugs available only in very small quantities, when these compounds were incubated with the cellular graft prior to innoculation into the recipients.

Methods

Hla: (F344xW) F_1 hybrid offspring from mating inbred male Fisher and female Wistar rats from a closed colony, where supplied by Hilltop Lab Animals (Chatsworth, California) and used as cell recipients. Parental Fisher or Wistar lymphocytes were usually injected into the plantar surface of the hind paw and the hybrid animals were killed 1 week later. For some experiments, lymphocytes were also injected into the front paws as well. Popliteal nodes (following hind paw injection) and spleens were removed and weighed after trimming off the fat. In addition, the lower abdominal nodes (especially the lumbar and inguinal nodes) and axillary nodes were also examined to determine if there was any significant increase in their size. Any splenic hypertrophy provided an index of a concurrent systemic reaction.

The injected lymphoid cells were obtained from the thymus, spleen, various nodes, blood and thoracic duct lymph. Their number was determined with a Coulter counter after lysing red cells with saponin and only the number of saponin-resistant cells are recorded hereafter. For routine experiments, spleen cells were preferrrd as the lymphocyte graft. When drugs were applied *in vitro* to these splenic lymphocytes, extraneous erythroid cells were first removed by washing the spleen cell population once with 9 volumes of isotonic ammonium chloride (0·87% w/v) buffered with one volume 0·15 M Tris HCl and then once with Hanks' medium after a rapid centrifugation to remove clumped (erythroid) cell membranes. Cells were checked for viability (trypan blue exclusion) and injected in 0·1 ml Hanks' medium per foot pad. Recipient animals were weighed before injection of the cells and again prior to killing. Drugs were administered according to schedules described in **Results** by oral intubation as suspensions in 3% gum arabic. Each experiment contained a control series of animals which received no drug and only the transferred spleen cells. In all cases the drug-treated animals and the control animals received the same number of spleen cells in order that adequate comparisons could be made. The regimen used for treatment was that Day 1 was the date of cell transfer and beginning of the experiment. Therefore, animals that were treated on Days 1, 2 and 3 were actually treated on the day of cell transfer and two subsequent days; those of Days 4, 5 and 6 were dosed on the 4th day, 5th day and 6th day after transfer, with all animals being killed 7 days after the day of transfer. For those animals that were treated on Day −2 and −1, the regimen was that 24 h before the date of either spleen removal or receiving of spleen cells was Day −1, Day −2 was 48 h prior to transfer of cells. Therefore, the animals had the last dose 24 h before the cells were transferred (donor being treated) or received (recipient being treated).

Compounds were applied to graft cells *in vitro* by incubating them with a suspension contained in 4 ml Hanks' medium and 1 ml 0·1 M sodium phosphate, pH 7·4 for 20 min at 37° C; cells were then isolated by slow centrifugation, resuspended in a small volume of Hanks' medium (usually 0·3 ml) and checked for viability by trypan blue exclusion before innoculation into two or more recipients. In these *in vitro* experiments, cells treated with drugs were innoculated into one hind paw of a F_1 hybrid while an equal number of similarly incubated, but untreated, cells were innoculated into the other hind paw; so that each animal was the subject of both a control (untreated) and an experimental (treated) reaction in each of its popliteal nodes.

Cyclophosphamide was activated *in vitro* by non-enzymic hydroxylation with ferrous ions and ascorbate (Udenfriend, Clark, Axelrod & Brodie, 1954).

Drugs

Azathioprine, chlorambucil and melphalan (Burroughs Wellcome, N.C.), dexamethasone, ethacrynic acid and indomethacin (Merck, N.J.), methotrexate (Lederle, N.Y.), phenylbutazone (Geigy, N.Y.), flazalone (Riker Labs, Minn.), cycloleucine and homocycloleucine (Wyeth Labs, Penn.), alanosine (Lepetit S.A. Italy), cyclophosphamide, isophosphamide and melengesterol (Mead Johnson, Ind.), EN-3638 (Endo Labs, N.Y.) Oxisuran (Warner-Lambert, N.J.), procarbazine, hydrochloride (Roche, N.J.), salicylazosulpha-pyridine (Pharmacia, N.J.); busulphan, gold sodium thiomalate and mechlorethamine hydrochloride (Aldrick Chem. Co., Wisc.), mannomustine (Berck, England); dibromomannitol (Schuchard, Germany), cycloheximide and mitocycin-C (N.B.C., Ohio).

Results

In vivo *studies*

Treatment of donor (Wistar) rats

The doses of azathioprine, dexamethasone and methotrexate used in the experiments of Table 1 were intentionally very high since it was not anticipated that these would affect those cells which would ultimately elicit a graft versus host

TABLE 1. *Effect of treating donor (W) rats with drugs for two days before transferring their splenic lymphocytes*

Experiments*	Popliteal node mg±S.E.M.	Popliteal and lumbar node mg±S.E.M.	Spleen/ B.W. (mg/g)
1 Control	153±4	222±11	5·6±0·5
Azathioprine 100 mg/kg	156±21	212±21	4·4±0·1
50 mg/kg	134±21	198±5	5·0±0·2
2 Control	69±7	94±7	3·3±0·1
Dexamethasone 125 mg/kg	44±4**	71±6	3·4±0·1
3 Control	184±19	254±21	5·6±0·2
Methotrexate 20 mg/kg	142±8	221±15	5·9±0·4
10 mg/kg	184±20	244±18	5·0±0·4

* The data presented in this Table are individual experiments done independently with their own controls. At least duplicate experiments were performed, each one using at least three rats and injections in both rear paws. Thus the numbers in this and subsequent charts represent at least the average of 4–6 popliteal nodes and three spleens and standard errors. The spleen/B.W. refers to mg spleen/g body weight of recipient animals 7 days post transfer.
** $P \leqslant 0{\cdot}05$ relative to control.

reaction. It is apparent that even these high doses did not produce a pronounced effect athough dexamethasone 125 mg/kg reduced the subsequent reaction in the host. The reason for the small size of the nodes in those recipients, of experiment 2, Table 1, was because those animals receiving dexamethasone had very small spleens and the controls were matched accordingly so that an equivalent number of spleen cells from both groups would be injected into the F_1 recipients.

Pretreatment of recipient (hybrid) rats prior to spleen cell injection

The effects of the same three drugs on the recipient animals are shown in Table 2, but in these experiments they were administered to the hybrid recipients before

TABLE 2. *Effect of pretreatment of recipient ($F \times W$) hybrid with drugs for two days prior to cell transfer*

Experiments*	Popliteal node	Popliteal and lumbar node	Spleen/ B.W.
1 Control	153±4	222±11	5·6±0·5
Azathioprine 100 mg/kg	83±17**		4·2±0·3
50 mg/kg	159±7	209±14	5·1±0·02
Dexamethasone 125 mg/kg	24±4**	39±7	2·3±0·2
2 Control	164±7	236±13	4·4±0·7
Methotrexate 20 mg/kg	163±6	232±5	3·5±0·3
10 mg/kg	173±12	241±17	3·3±0·3

* See footnote to Table 1. ** $P \leqslant 0{\cdot}05$ relative to control.

the Wistar graft cells were injected. There are two major differences to be seen by comparison of Table 2 with Table 1. First, azathioprine at 100 mg/kg inhibited the graft versus host reaction initiated with normal spleen cells and secondly, dexamethasone at the dose of 125 mg/kg virtually abrogated the graft versus host reaction. This dose of dexamethasone had no effect on the size of the local nodes in animals not challenged with a graft, as measured 8 days after withdrawing this drug. The effect of the latter drug is apparent not only on the size of the popliteal and lumbar nodes, but also on the spleen to body weight ratio which is approximately one half of that seen in the controls and is in the range of normal rats. Neither methotrexate (20 mg/kg) nor azathioprine (50 mg/kg) had any inhibitory effect on the subsequent graft versus host reaction elicited by normal spleen cells in these pre-treated recipients.

Treatment of recipient (hybrid) rats on the day of cell transfer and for the subsequent two days

In Table 3 there are five experiments in which the action of azathioprine, dexamethasone and methotrexate were combined with that of some other compounds reported to be either anti-inflammatory or immunosuppressive drugs. In this concurrent treatment compounds were found to inhibit the graft versus host reaction whereas when given prophylactically (Tables 1 and 2) they did not. When these data were plotted graphically, the ID_{50} for methotrexate was found to be 1·5 mg/kg. Similarly for dexamethasone, doses of which as low as 0·3 mg/kg profoundly inhibited the reaction, the ID_{50} dose was 0·5 mg/kg. Azathioprine 60 mg/kg showed as much, or more, inhibition of the graft versus host reaction in the popliteal node (Table 3) as did 100 mg/kg when given to the recipient rat prior to the transfer of cells (Table 2). The immunosuppressive amino acid analogue

TABLE 3. *Effect of daily dosage of drugs for Day* 1, 2, 3 *on development of GvHR in* F_1 *hybrid recipients*

	Drug (mg/kg)*	Popliteal node	Popliteal and lumbar node	Spleen/ B.W.
1	Control	145±17	194±18	4·1±0·1
	Azathioprine 60	65±8**	92±10	3·9±0·4
	Indomethacin 3	108±7**	149±8	5·3±0·9
	Phenylbutazone 100	100±9**	146±11	3·7±0·1
	Flazalone 100	110±11	156±15	3·3±0·3
2	Controls	82±5	123±4	3·1±0·5
	Dexamethasone 1	28±8**	41±10	2·5±0·3
	Dexamethasone 0·3	47±6**	62±5	2·9±0·3
	Dexamethasone 0·1	79±8	108±7	3·2±0·1
3	Controls	140±9	176±11	4·2±0·3
	Cycloleucine 100	25±3**	43±3	2·8±0·1
	Cycloleucine 50	51±4**	73±14	3·4±0·1
	Cycloleucine 25	92±7**	129±7	3·2±0·1
	Homocycloleucine 50	136±16	180±13	4·0±0·4
4	Control	111±7	175±13	4·1±0·3
	Methotrexate 1	60±2**	87±5	4·0±0·2
	0·5	88±5**	119±10	3·7±0·3
5	Control	215±12	281±20	3·8±0·2
	Alanosine 20	159±30	247±30	4·9±0·7

* See footnote to Table 1. ** $P \leqslant 0{\cdot}05$ relative to control.

cycloleucine showed an ID_{50} of 35 mg/kg. Homocycloleucine (1-amino cyclohexanecarboxylic acid) which is chemically similar to cycloleucine, showed no inhibition whatsoever at 50 mg/kg. The anti-inflammatory compounds indomethacin, phenylbutazone and flazalone, exhibited modest inhibition of the nodal enlargement. The doses used for these non-steroid anti-inflammatory drugs were high and these responses are not considered to be very important biologically.

Treatment of recipient (hybrids) rats on the fourth, fifth and sixth day after spleen cell transplant

The data reported in Table 4 essentially amplify those in Table 3. Dexamethasone and methotrexate are both very active compounds in inhibiting the later stages of the graft versus host reaction. For dexamethasone, the ID_{50} is

TABLE 4. *Effect of daily dosage for Day* 4, 5, 6 *post-transfer on development of the graft versus host reaction in* F_1 *hybrids*

	Experiments*	Popliteal node	Popliteal and lumbar node	Spleen/ B.W.
1	Control	122±23	156±27	4·0±0·2
	Dexamethasone 1 mg/kg	21±7**	33±9	2·6±0·3
	,, 0·3	46±8**	61±3	3·1±0·7
	Methotrexate 2	47±11	80±15	2·9±1·0
	,, 1	62±10	93±9	3·4±0·3
	Cycloleucine 100	43±7**	65±3	3·1±0·1
	,, 25	62±10	108±24	3·7±0·1
2	Control	107±18	164±25	4·0±0·5
	Phenylbutazone 100	60±5**	102±12	4·6±0·7
	Indomethacin 3	75±8	140±12	5·4±0·3
	Azathioprine 60	53±9**	114±9	3·8±0·1
	Flazalone 100	64±6	113±8	4·2±0·2
3	Control	215±12	281±20	3·8±0·2
	Alanosine 20	136±8**	195±18	3·8±0·3
4	Control	153±47	202±14	3·9±0·4
	Chlorambucil 7·5†	92±12	154±21	1·9±0·3
	Cyclophosphamide 15	82±10	117±22	2·3±0·1
	Isophosphamide 30	81±25	115±26	2·0±0·1

* See footnote to Table 1. † Higher doses were toxic (loss of weight).
** $P \leqslant 0{\cdot}05$ relative to control.

approximately 0·6 mg/kg and for methotrexate, the ID_{50} is 2 mg/kg. Cycloleucine had an ID_{50} of approximately 25 mg/kg. Azathioprine at 60 mg/kg again demonstrated anti-inflammatory/antiproliferative activity. Non-steroid drugs phenylbutazone, indomethacin and flazalone again at the high doses used, demonstrated some degree of anti-inflammatory/antiproliferative activitiy.

If the data in Table 3 and 4 are combined, it appears as though all these drugs are much more active when given after the transfer of spleen cells and initiation of the graft versus host reaction than at earlier periods (see Tables 1 and 2). Experiments 3 and 4 of Table 4 illustrate the efficacy of a new drug alanosine (Arrigoni-Martelli, Schiatti & Silva, 1971) and of some alkylating agents given therapeutically.

Treatment of recipient (hybrid) rats for seven days

With this schedule, drugs were administered orally on the day of transfer (immediately following the transfer) and for the subsequent 6 days once daily to groups of 4 or more rats, yielding at least 8 (or 16) sets of leg nodes with which to assess the drug efficacy.

TABLE 5. *Effect of treating recipient (F_1 hybrid) rats once daily for 7 days with some putative immunosuppressant drugs*

	mg/kg	Node weight as % of GvHR control*	Spleen weight/ body weight (mg/g)
No drug	—	100	3·5–4·2
Cyclophosphamide	10	11	2·2
Cycloleucine	20	49	3·6
Alanosine	10	41	3·0
EN-3638	100	40	2·7
Oxisuran	100	62	3·1
ICI-47,776	10	62	3·7
Procarbazine HCl**	30	80	3·3
Salicyl-azosulphapyridine	100	93	3·2
Mechlorethamine HCl**	0·25	90	4·4
Melengesterol††	5	80	3·3
Phenylbutazone	100	48	3·1
Na Aurothiomalate†	8	93	4·3

* Control = equal number of viable cells given to undrugged animals.
** Higher doses causing significant weight loss and were considered toxic.
† Given daily i.m.
†† Given on days 1, 2, 3. (When given on days 4, 5 and 6 it was inactive.)

Table 5 compares some novel immunosuppressant drugs with cyclophosphamide and cycloleucine. The possibility that phenylbutazone has some intrinsic lymphoid-depressant activity, beyond its anti-inflammatory activity, is also indicated here as well as in Table 3 and 4. By contrast anti-arthritic gold preparations (administered intramuscularly) were devoid of activity in this assay. At the single doses used in this experiment, the only compounds showing 50% or greater inhibition without serious toxicity were cyclophosphamide, cycloleucine, alanosine, EN-3638 and phenylbutazone.

In vitro *studies*

Throughout this section the term 'lymphocyte deactivation' is used to describe the *in vitro* effect of a drug in rendering graft cells much less competent for eliciting a graft versus host reaction, without overall loss of viability.

Alkylating agents

Thiol-blocking agents such as maleimide, ethacrynic acid (0·1 mM) and *p*-hydroxy and *p*-chloro mercuryphenylsulphonates (Table 6) deactivated lymphocytes *in vitro* in short term incubations, at fairly high drug concentrations (0·1–0·5 mM) without rendering them less viable. By contrast, active nitrogen mustards such as mechlorethamine (HN2), chlorambucil and melphalan were rather potent lymphocyte deactivating agents *in vitro*, though both these drugs were effective inhibitors

TABLE 6. *Effect of some drugs* in vitro *on the capacity of the graft cells to elicit a subsequent graft versus hybrid reaction in F_1 hybrids*

Drug	Concentration μM	GvHR as % of control*
Cyclophosphamide	2000	98
Isophosphamide	3,500	83
Mechlorethamine HCl	2	32
Chlorambucil	10	38
Melphalan	10	33
Mannomustine	500	38
Busulfan	1,000	39
Azathioprine	1,000	72
1,6-Dibromomannitol	1,000	100
Methotrexate	1,000	100
Maleimide	50	24
Cycloheximide	100	75
p-Chloromercuryphenylsulphonate	500	13
Mitomycin-C	500	10
ICI-47,776**	100	67

* As elicited in the other hindlimb of the same animals by undrugged cells.
** i.e. 3-Acetyl-5-(*p*.fluorobenzylidene)-2·5-dihydro-4-hydroxy-2-oxothiophen.

of the graft versus host reaction when administered to the F_1 recipients (see Table 4, 5). Potent graft-deactivating products were obtained by *in vitro* pre-activation of cyclophosphamide, by non-enzymic hydroxylation on incubating with $FeSO_4$-ascorbate-Na_2 edetate at pH 5·4 for 90 minutes, followed by subsequent neutralization to pH 7·4 (for incubation with the graft cells): Omission of ferrous ions or ascorbate or cyclophosphamide from the mixture resulted in products with minimal or no lymphocyte-deactivating activity. The very potent effect of mechlorethamine *in vitro* could be largely prevented by a brief prior incubation of the cells with sodium thiosulphate (5 mM), provided the concentration of mechlorethamine did not exceed 5 μM with cell concentrations of $30–50 \times 10^6$ cells/ml. The *in vitro* potency of chlorambucil (20 μM), but not of maleimide (100 μM), was likewise attenuated by 1 mM thiosulphate.

Other immunosuppressant and anti-inflammatory drugs

With the exception of mechlorethamine, chlorambucil (see above) and ICI-47,776 (Davies, 1968), none of the other compounds listed in Tables 1 to 5 had a significant effect on the graft cells *in vitro* at concentrations of 0·5 mM or at saturation (if 0·5 mM was not attainable in neutral aqueous salt solutions). A structural isomer of ICI-47,776=1-acetyl-4*p*. fluorophenyl-2-hydroxy-5-oxo-3-thiocyclopentene formed by rearrangement in alkali or u.v. light (O'Mant, 1968) was devoid of activity. Compounds such as flazalone (0·5 mM), flufenamic acid (0·3 mM), or corticosteroids, e.g. hydrocortisone hemisuccinate (0·5 mM), known to have profound but reversible effects on lymphocyte metabolism (Whitehouse, 1971 ; Famaey

& Whitehouse, 1973 ; Darzynkiewicz & Pienkowski, 1969) proved to have no lasting effect on the graft cells as far as their ability to initiate the full graft versus host reaction. However, boiled solutions of flazalone HCl (R-760) generated lymphocyte-deactivating products.

Physical and chemical inactivation of graft cells

Exposure of the graft cells to varying quantities of X-irradiation indicated an ID_{50} above 500 rad (Table 7). Likewise, exposure to temperatures above 41° C led to rapid loss of graft competence. Cells were profoundly deactivated by periodate oxidation, from which they could be reactivated by subsequent reduction with borohydride (Zatz, Goldstein, Blumenfeld & White, 1973), indicating that chemical manipulation of the cell surface coat caused a prolonged deactivation of these graft lymphocytes.

TABLE 7. *Effect of some physical and chemical manipulations on the capacity of graft cells to elicit a subsequent graft versus host reaction (GvHR) in F_1 hybrids*

Treatment	% viability	GvHR as % control
None	95	100
Sonication (1 min)	0	5
X-irradiation—100 rad	90+	88
—500 rad	90+	55
—1,000 rad	90+	41
—4,000 rad	90+	26
Incubation at 37°	97	110
41°	85	92
45°	50	25
56°	2	21
$NaIO_4$	80	8
$NaBH_4$	90	95
$NaIO_4$ then $NaBH_4$ (10 min)	90	62

Cells were incubated for 15 min either at elevated temperatures or with 1 mM $NaIO_4$ at pH 7.4 and 21° C, then with 2 mM $NaBH_4$ for 10 min at 21° C. Cells were kept at 4° C for transfer to, and from, the radiation facility.

Discussion

In a previous study (Levy, 1973) it was found, by the use of the Simonson assay of splenomegaly in the mouse, that drugs such as cyclophosphamide or azathioprine when given to donor animals did not inhibit the (systemic) graft versus host reaction in adult F_1 hybrid mice. These drugs, however, were effective in the F_1 recipient mice as indicated by decreased splenomegaly. The localized graft versus host reaction in the rat offered advantages for studying drug action that are of some general interest. The assay itself is in a larger species with a generally slower rate of drug metabolism and the assay is much more sensitive in the degree of response. For example, the splenomegaly observed in the F_1 recipient mice after grafting parental spleen cells is usually between 3 and 5 times normal size and requires two weeks for optimal development. However, in the popliteal node assay in the rat, the degree of change in node size attained within one week only, is in a range between 20 and 50 times that of the control in size. Apart from the mechanical and logistic aspects of utilizing another graft versus host reaction for the evaluation of drugs, this particular test offers some reasonable theoretical advantages over other studies. As we have illustrated here, we can study the effects of both physical and chemical treatments of the graft cells *in vitro*

prior to their administration to the animal. The target organs are pairs of nodes so that one of the pair can serve as a control for the other. We can be reasonably assured from viability studies that the graft population includes only live cells and therefore that doses of drugs used *in vitro* will be only those which are not immediately toxic to the cells *in vitro*. The possible role of a drug metabolite in deactivating immunocompetent cells *in vivo* becomes even more apparent when a combination of *in vitro* and *in vivo* studies is used ; thus cyclophosphamide is active *in vivo* but not *in vitro* as shown in the present experiments.

The so-called ' graft versus host reaction ' is actually a complex set of reactions induced by non-immunogenic viable graft cells recognizing in the host foreign antigens and reacting to these foreign antigens. In the intact adult animal this graft reaction is compounded by the fact that the host is also capable of reacting to the antibody-producing lymphoid graft cells. Not only is this evident from studies which we have performed using this particular combination of parent and hybrid rats, but early studies by Fox & Howard (1963) with a mouse graft versus host reaction indicate that, despite the fact that the graft cells can reside and propagate within the host, the lymphoid hypertrophy (that is characteristic of the reaction) regresses with time. In this specific case (using splenomegaly in the mice as an index of graft versus host reaction) chromosomal analysis indicated residual, viable graft cells during the period when the splenomegaly disappeared. These graft cells are either no longer capable of continuing to provide a stimulus to the recipient or the recipient is now neutralizing whatever it is the graft cell contributes to initiate (or perpetuate) the graft versus host reaction. In the manifestation of a graft versus host reaction there is always a proliferative response which is predominantly that of the host's tissues, i.e., not of the donor's tissue (Volkman, 1972). Thus using the graft versus host reaction, drugs can be studied for their effects on (a) the ability of the graft to react against the host (e.g., making an anti-host antibody) with all its complexities, and (b) the host's (mainly proliferative) response to the grafted lymphocytes or their secretions. The drugs that we have investigated appear to be much more effective as anti-proliferative drugs than they are as immunosuppressive agents since pretreatment of the donor animals with some of these drugs *in vivo* causes very little loss of immunocompetence, as measured by ability to subsequently elicit a graft versus host reaction, except with extremely high doses of drug.

Treatment of these competent cells *in vitro*, especially with irreversibly acting (e.g. alkylating agents), may inhibit the subsequent graft versus host reaction. Any dichotomy between these *in vitro* and *in vivo* drug studies certainly requires further study. It is obvious that drug metabolism can generate biologically effective agents as well as inactivate compounds which are effective *in vitro*. Use of the combined methods of drug treatment of cells with drug treatment of the whole host animal (recipient) provides an opportunity to evaluate the role of drug metabolism in determining bioefficacy, with a single parameter.

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