STUDIES ON THE IMMUNOTHERAPY OF RUNT DISEASE IN RATS*

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When animals are inoculated with lymphohematopoietic cells of homologous origin which, for one reason or another, they are unable to reject, they may be stricken with an often fatal, wasting syndrome, usually referred to as runt, transplantation, or homologous disease (1–3). The essential features of this syndrome include loss of weight or retardation of growth, diarrhea, a diffuse dermatitis, hypertrophy of lymphoid organs followed by atrophy of these organs, atrophy of the thymus, and dyscrasia of the blood and bone marrow (4, 5). Although extensive analyses of this syndrome in rodents and chickens have shown that it is caused by an immunologic response on the part of the donor's immunologically competent cells against alien transplantation antigens of the host (i.e., it is a graft-versus-host [GVH] reaction), its pathogenesis is still very incompletely understood. The possible ancillary role of microbial organisms of endogenous or exogenous origin in the immunologically debilitated hosts is still undecided.

A necessary condition for the development of GVH disease is incompatibility between donor and host at the major histocompatibility locus for the species concerned (e.g., the H-2 locus in mice or the Ag-B locus in rats) (3), and its severity depends principally upon the number of small lymphocytes present in the cellular inoculum, thymocytes being relatively innocuous (6).

Although, initially, homologous disease was regarded as a laboratory artifact, its occasional occurrence in man is now recognized as a consequence of either (*a*) a natural maternal → fetal transplacental "transfusion" (7), (*b*) a therapeutic lymphocyte-containing cellular transfusion of fetuses or infants (8, 9), or (*c*) transplantation of bone marrow in the treatment of hematopoietic failure and leukemia (10). Thus, apart from any possible light they may shed upon the pathogenesis of homologous disease, studies on its prevention or arrest have clinical relevance.

In mice, the course of runt or homologous disease can be altered by administration, just before or soon after injection of the initiating homologous cellular inoculum, of a variety of agents including: (*a*) suspensions of isologous lymphoid cells from normal adult donors; (*b*) lymphoid cells or antiserum from isologous donors specifically sensitized against tissues of the putative attacking cells; (*c*) isoantiserum specifically directed against the antigens of the *host* under attack and raised in animals of the homologous strain providing the attacking cells; and (*d*) immunosuppressive agents (11–17).

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Although the pathology of runt disease has been more thoroughly studied in rats than in mice, few therapeutic studies have been performed in the former species. This is unfortunate since in infant mice the clinical indications of certain bacterial and viral infections simulate those of runt disease so closely as to complicate interpretation of the results of studies designed to prevent this disease (3). With rats, on the other hand, the highly pathognomonic status of the skin lesions associated with runt disease in certain strain combinations obviates this complication. The purpose of this communication is to present the results of a comparative study of the capacity of injections of normal and sensitized isologous adult lymph node cells, on the one hand, and of isoantisera and heterologous rabbit anti-rat lymphocyte serum, on the other hand, to prevent the development of, or abort, an acute attack of runt disease in this species.

Materials and Methods

Rats.—Domestically maintained Lewis (Ag-B^1) and BN (Ag-B^3) isogenic strains provided donors and recipients respectively. Previous work had shown that intravenous inoculation of neonatal BN hosts with 10–20 million lymph node cells from normal adult Lewis donors causes acute runt disease that is invariably fatal within 20 days (4). The first indication of the disease is thickening and tightness of the skin on about the 10th day, frequently associated with erythrodermia of the extremities. This is rapidly followed by a generalized exfoliative dermatitis, progressive increase in rigidity of the integument to the point of considerable immobilization of the limbs, arrest of weight gain, and hypothermia.

The Disease-Inciting Inoculum.—A suspension of 20×10^6 Lewis lymph node cells, prepared from the axillary, brachial, cervical, and mesenteric nodes, and dispensed in Hanks' balanced salt solution according to standard procedures described elsewhere (18) was employed as the standard, disease-inciting inoculum in this study. This was administered intravenously in a standard volume of 0.1 ml to hosts less than 24 hr old. Since this treatment causes 100% mortality, the effectiveness of the therapeutic agents injected in conjunction with, or at various times before or after the "attacking" cells, was assayed in terms of their ability to save or prolong the subjects' lives.

Therapeutic Inocula.—

(*a*) Lymph node cell suspensions were prepared from normal adult BN rats, or from BN rats sensitized by initial bilateral homografts of Lewis skin followed by a booster intraperitoneal injection of $200–400 \times 10^6$ Lewis lymph node and spleen cells.

(*b*) Isoantisera were obtained from BN or DA rats given a primary Lewis skin graft on their right thoracic wall followed, about 10 days later, by a similar graft contralaterally. These animals subsequently received a booster intraperitoneal inoculation of Lewis lymphoid cells 7 days before bleeding for serum.

(*c*) Heterologous anti-lymphocyte serum (ALS) was raised by injecting adult New Zealand rabbits intravenously with thymic cells prepared from DA female weanling rats, according to the procedure of Levey and Medawar (19, 20). After decomplementation, the serum was absorbed with rat erythrocytes, sterilized by filtration, and stored in the deep freeze. The hemagglutinin titer of this antiserum against rat erythrocytes was $> 1:1600$. The lymph agglutinin titer against suspensions of rat lymph node cells was 1:800.

All treated animals that survived to adulthood were challenged with Lewis skin homografts to determine whether their immunologic reactivity had been affected either by the putative attacking cell inoculum or by the therapeutic treatment.

Intravenous inoculations were always via the anterior facial vein (18). When two inoculations were required by this route, the veins on opposite sides of the face were employed.

RESULTS

Control Data: Effect of Standard Inoculum of Lewis Lymph Node Cells.—To evaluate the protective influence of the various agents on runt disease, putative therapeutic treatment was withheld from a few members of each litter of neonatal BN rats inoculated with 20 million Lewis node cells. As anticipated from the previous findings with this strain combination (4), all of a total of 46 BN control animals displayed the first clinical signs of the disease within 11–15 days and all succumbed within 13–18 days. A similar fate overtook an additional group of nine animals which received their Lewis cell inocula via the intraperitoneal route (see Table I, Experiments 1 and 2).

Influence of Administration of Isologous Node Cells from Normal Donors on the Development of Runt Disease.—Since the development of runt disease in the context of the present study depends upon the inability of the hosts to reject the antigenically foreign cells, through immunologic immaturity and/or the development of tolerance of them, it was assumed that transfusion of BN recipients with lymphoid cells from *normal* adult BN rats would furnish them with a ready-made, functionally developed, normal immunologic response mechanism, i.e., an adoptive protective mechanism. Accordingly, panels of BN rats which had received 20×10^6 Lewis node cells intravenously within 24 hr of birth were inoculated with a similar number of BN node cells, either at the same time, or after delays of 24, 48, or 72 hr, respectively.

The results, summarized in Table I, show that whereas virtually complete suppression of runt disease was accomplished when the BN cells were given immediately following the Lewis cells via a different vein (Table I, Experiment 3), there was no discernible protection if their administration was delayed by 24 hr or longer (Table I, Experiments 7, 9, 10). Even doubling the number of BN cells injected after a delay of only 24 hr failed to impair significantly the development or progress of the disease (Table I, Experiment 8). The most striking indication of the critical importance of the timing of administration of the BN cells was the finding that a delay of as little as 4 hr considerably impaired the protection conferred, as evidenced by the 85% incidence of runt disease and the 60% mortality (Table I, Experiment 5).

In view of this surprising observation, the influence of prophylactically treating neonatal BN hosts intravenously with 20×10^6 isologous node cells from normal adult donors 4 hr *before* intravenous inoculation with 20 million Lewis lymphoid cells was investigated. The results (Table I, Experiment 6) do not differ significantly from those reported above, and provide a further indication of the critical importance of the time of administration of isologous normal adult BN cells for production of any beneficial influence.

If, instead of inoculating the Lewis node cells and the BN node cells via different veins at approximately the same time, the two inocula were first mixed in vitro and then inoculated intravenously, the protective effect was practically as good as that obtained when the two cell suspensions were injected separately (Table I, Experiment 4).

These findings show that intimate exposure of the BN cells to the Lewis cells before injection is not essential for them to confer protection upon the host. However, this certainly does not exclude the possibility that some kind of inti-

TABLE I

Influence of Time and Manner of Administration of a Standard Inoculum of Node Cells from Normal BN Donors into Infant BN Hosts on Their Capacity to Modify the Course of Runt Disease Initiated by Injection of 20 Million Lewis Lymph Node Cells

Exp.	Route of administration of potentially harmful Lewis node cells	Therapeutic BN node cells administered			Incidence of runt disease		Time of appearance of symptoms		Mortality		Survival time	
		No. $\times 10^6$	Time of administration after injection of Lewis cells	Route			Range	Median			Range	Median
			hr			%	*days*	*days*		%	*days*	*days*
1	i.v.	None*			46/46	100	11–15	12	46/46	100	13–18	16
2	i.p.	"			9/9	100	12–14	13	9/9	100	14–20	16
3	i.v.	20	0	i.v.	1/12	8	20		0/12			
4	i.v.	20	0	i.v.‡	2/13	15	14, 17		1/13	8	19	
5	i.v.	20	+4	i.v.	11/13	85	12–16	13	8/13	60	15–25	16
6	i.v.	20	−4	i.v.	9/11	82	12–13	13	8/11	73	15–18	18
7	i.v.	20	+24	i.v.	10/10	100	12–13	12	10/10	100	14–16	15
8	i.v.	40	+24	i.v.	18/18	100	12–15	13	18/18	100	14–26	17
9	i.v.	20	+48	i.v.	7/7	100	13–16	14	7/7	100	18–21	18
10	i.v.	20	+72	i.v.	15/15	100	11–14	12	15/15	100	14–17	15
11	i.v.	20	0	i.p.	9/14	64	12–14	12	9/14	64	15–17	15
12	i.p.	20	0	i.v.	18/18	100	12–15	13	16/18	89	15–22	16

* Controls.

‡ Mixed with Lewis cells before injection.

mate relationship may have to be established between the two cell types in vivo to invoke a beneficial immunological response on the part of the BN cells. If this is the case, then Haller's (21) evidence suggests that the spleen might be the organ in which such a confrontation takes place, since this seems to be the tissue in which a large proportion of intravenously injected lymphoid cells rapidly become localized in neonatal murine hosts.

If such confrontations are important, then administration of the two cell populations by physiologically different routes should reduce their incidence, or delay their occurrence, and thereby diminish the protective effect of the isologous lymph node cells. To investigate this possibility, one panel of neonatal BN rats received the standard inoculum of Lewis node cells intravenously, followed

immediately by the same number of BN node cells intraperitoneally (Table I, Experiment 11). In a second panel of neonatal BN hosts, the routes of inoculation of the isologous and homologous cell suspensions were reversed, and the BN cells were introduced directly into the blood stream (Table I, Experiment 12). The results are consistent with the thesis that some kind of prompt in vivo cellular confrontation is required to abort the adverse effect of the homologous cells, for in both panels there was a high incidence of the disease, and the majority of affected animals succumbed. However, contrary to what might have been expected, the incidence of the disease was significantly lower when the BN cells were introduced intraperitoneally, and the Lewis cells intravenously, rather than in the converse situation (64% vs. 100%: $0.05 > P > 0.02$).

TABLE II

Ability of Sensitized BN Lymph Node Cells to Modify Course of Runt Disease When Given at Various Times after the Inoculation of 20 Million Lewis Lymph Node Cells into Newborn BN Rats

Exp.	BN node cells administered		Route	Incidence of runt disease		Time of appearance of symptoms		Mortality		Survival time	
	No. $\times 10^6$	Time of administration after injection of Lewis cells				Range	Median			Range	Median
		hr			%	*days*	*days*		%	*days*	*days*
1	20	+3	i.p.	4/13	30	12–14	—	1/13	8	26	—
2	20	+4	i.v.	14/14	100	11–13	12	13/14	93	14–24	15
3	20	+4	i.p.	10/10	100	11–13	12	8/10	80	15–17	16
4	20	+5	i.v.	7/8	88	11–14	13	4/8	50	15–16	—
5	20	+5	i.p.	5/5	100	13	13	5/5	100	15–17	16
6	20	+6	i.p.	12/12	100	11–13	12	12/12	100	14–17	15
7	40	+6	i.p.	10/10	100	11–12	12	10/10	100	14–15	15

Influence of Administration of Isologous Node Cells from Sensitized Donors on the Development of Runt Disease.—Since it was anticipated that lymphoid cells from BN donors specifically sensitized against Lewis tissues would exert a greater degree of protection than cells from unsensitized donors, it seemed reasonable to delay their administration for at least 3 days after the BN hosts had received their standard neonatal inoculum of 20 million Lewis cells. The findings vindicated this decision since only 4 of 13 BN rats which received a protective inoculum of 20×10^6 BN anti-Lewis lymph node cells by the intraperitoneal route, 3 days after the intravenous injection of Lewis node cells, showed symptoms of the disease and only one of these animals died (Table II, Experiment 1). However, when administration of the protective inoculum was delayed until the 4th, 5th, or 6th day, its effectiveness was very significantly reduced. At 4 or 5 days (Table II, Experiments 2–5), only 7 of 37 animals were

saved and none survived when treatment was given on the 6th day (Table II, Experiment 6). Indeed, even 40 million BN cells at this time failed to alter the course of the disease (Table II, Experiment 7). Moreover, the route by which the sensitized BN cells were given at 4 or 5 days, i.e., whether inoculated intravenously or intraperitoneally, apparently made little, if any, difference. The influence of administration of BN cells by the intravenous route to 6 day old

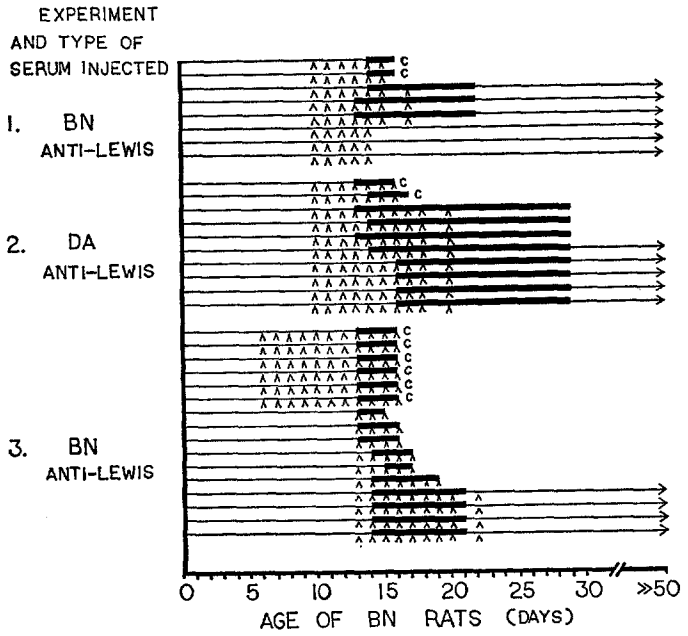

FIG. 1. Illustrating protective influence of administration of antiserum to BN rats injected intravenously with 20×10^6 Lewis lymph node cells on the day of birth.

The life-span of each subject is indicated by a horizontal line. Thickened lines indicate presence of cutaneous lesions characteristic of runt disease. Time of death is indicated by termination of the lifelines. Arrow heads along the lifelines indicate days of injections of standard dose of 0.2 ml of antiserum. C indicates that animal received serum from normal BN donor and is therefore a control.

BN hosts was not tested because of the difficulty of carrying out intravenous injections in rats of this age.

None of the BN hosts receiving a neonatal inoculation of Lewis cells, and surviving as a result of the protective effect of a subsequent inoculation of isologous BN node cells, from either normal or sensitized donors, gave any evidence of being tolerant of Lewis test skin homografts.

Capacity of Isoantisera to Alter the Course of Runt Disease.—Since the

results of Křen and his associates (22, 23) indicated that isoantisera may be more effective in inhibiting GVH reactivity than sensitized lymphoid cells, the protective effect of antiserum with the present Lewis and BN rat strains has been investigated.

In all the experiments to be described, the BN subjects received a normally lethal intravenous injection of 20×10^6 Lewis node cells within 24 hr of birth as before. Serum was inoculated by the intraperitoneal route.

In the first experiment (Fig. 1), each of six 10 day old BN subjects received five consecutive daily injections of 0.2 ml of BN anti-Lewis antiserum. On completion of this treatment, three of them looked so healthy that no further serum was administered. Since the remaining three animals looked less healthy, and exhibited some exfoliation of their skins, they received two additional injections of the antiserum, on days 15 and 17. All six animals grew up normally. That serum from normal, unsensitized BN donors has no protective effect was evidenced by the fact that two littermates of the above animals treated with it died of typical runt disease on the 16th day.

When DA rats (Ag-B^4) are immunized with Lewis tissue antigens, the cytotoxic and hemagglutinating antibodies that appear are known to be principally directed at antigens determined by the Lewis Ag-B^1 allele. A DA anti-Lewis isoantiserum should, therefore, confer protection against the ravages of runt disease upon infant BN rats injected with Lewis lymphoid cells. To test this prediction (Fig. 1, Experiment 2), eight 10 day old BN rats received daily injections of 0.2 ml of DA anti-Lewis serum from days 10 to 18, and a final injection on day 20. Although all of these animals had characteristic skin lesions when they were 16 days old, and in six the symptoms of runt disease were severe, three animals survived to 29 days, and five survived indefinitely, after recovering slowly from a severe retardation of their growth. Again, a similar regimen of treatment of two littermates with normal serum failed to alter the progress of the disease, these animals dying on the 16th and 17th days, respectively.

Finally, to get some idea of the maximal delay in initiation of antiserum administration compatible with a therapeutic influence on runt disease, 10 13 day old BN hosts were injected daily with BN anti-Lewis serum, until they either succumbed, or attained the age of 22 days. After this, no further serum was given (Fig. 1, Experiment 3). Although all subjects manifested some tautness or lesions of the skin when this treatment was initiated, and six died when they were 15–19 days old, four lived to adulthood, after considerable retardation of their growth. Compelling evidence that the protective effect was immunologically specific was provided by the observation that all 6 BN rats injected daily with normal BN serum, from 6 days of age, died on the 16th day.

Clearly, antiserum at the dosage levels administered is a much more effective therapeutic agent than isologous lymphoid cells from normal, or even specifi-

cally sensitized isologous adult donors. Indeed, antiserum can even arrest the course of the disease when first given at the time its symptoms have become overt.

None of the survivors of these various experiments gave evidence of being tolerant of Lewis tissues when challenged with a Lewis skin homograft.

Capacity of Rabbit Anti-Rat Lymphocyte Serum to Alter the Course of Runt Disease.—Antiserum raised in one species against lymphoid cells of another species (heterologous anti-lymphocyte serum, or ALS) has been shown to be a relatively harmless, but extremely effective, agent for suppressing the capacity

TABLE III

Influence of Injecting Normal Rabbit Serum or Rabbit Anti-Rat Thymocyte Serum into Infant BN Rats Inoculated at Birth with 20 × 10^6 Lewis Lymph Node Cells

Exp.	Type of serum	Serum treatment		Animals developing runt disease per No. injected	Time of appearance of symptoms of runt disease	Survival times of animals, *days*				
		Dosage per inoculation	Time of inoculation after birth			10–20	21–30	31–40	41–50	>50
		ml	*days*		*days*					
1	Immune	0.05	5,7	5/11	17–19	1	3			7
	Normal	0.05	5,7,9,11	6/6	12–13	6				
2	Immune	0.05	6,8,10,12	2/6	21–23	—	1			5
	Normal	0.05	6,8,10,12	1/1	13	1				
3	Immune	0.1	8,10,12,14	9/9	11–18	3	3	1		2
	Normal	0.1	8,10,12,14	2/2	11–12	2				
4	Immune	0.1	10,12,14,16	6/6	11–12	6				
	Normal	0.1	10,12,14,16	3/3	11–12	3				
5	Immune	0.1	10,11,12,13	8/8	11–14	4	4			
	Normal	0.1	10,11,12,13	4/4	11–14	4				
6	Immune	0.2	13,14,15	5/5	12–13	5				
	Normal	0.2	13,14,15	2/2	12–13	2				

of members of the lymphoid cell donor species to react against skin homografts (24, 25). Furthermore, treatment with ALS can abolish immunological memory in animals that have rejected first-set homografts (25), as well as abrogate the capacity of lymphoid cells from mice or rats to incite GVH reactions when inoculated into potentially susceptible homologous hosts (26–28). These and other findings, such as the ability of ALS treatment of guinea pig hosts to inhibit both normal and immune lymphocyte transfer reactions (29)—both expressions of GVH reactivity—constitute a strong prima facie case that rabbit anti-rat thymocyte serum should afford protection in infant rats otherwise destined to develop and succumb to GVH disease. The work now to be described was carried out to investigate this possibility.

Neonatal BN rats were injected intravenously with a standard dosage of

20×10^6 adult Lewis donor lymph node cells as before. Then, commencing 5 or 6 days later, the animals received two or four daily subcutaneous injections of 0.05 ml of ALS. To provide controls some littermates, which had also received Lewis lymphoid cells at birth, received decomplemented normal rabbit serum instead of ALS. The results (Table III, Experiments 1 and 2) show that this therapy was highly effective since it saved the lives of 12/17 (70%) of the subjects, some of whom had manifested unmistakable signs of runt disease after the

TABLE IV

Comparison of Optimal Protective Effects of Treating BN Rats Injected with 20 × 10^6 Lewis Node Cells with (a) Normal Isologous Adult BN Node Cells, (b) Node Cells from Adult BN Rats Sensitized against Lewis Tissue Antigens, (c) Isoimmune Anti-Lewis Serum, and (d) Heterologous Rabbit Anti-Rat Thymocyte Serum

Treatment given to infant BN rats			Proportion of animals which survived	
Therapeutic agent		Time(s) of administration of therapeutic agent		
				%
20 × 10^6 normal BN node cells		−4 hr	3/11	27
		0 "	12/12	100
		+4 "	5/13	38
		+24 "	0/28	0
20 × 10^6 BN anti-Lewis node cells		+3 days	12/13	93
		+4 to 5 "	7/37	19
		+6 "	0/22	0
BN anti-Lewis serum 0.2 ml		Daily +10 to +17	6/6	100
		Daily +13 to +22	4/10	40
Rabbit anti-rat thymocyte serum	0.05 ml	Days +6, +8, +10, +12	5/6	83
	0.1 ml	Days +8, +10, +12, +14	2/9	22
	0.1 ml	Days +10, +11, +12, +13, or +12, +14, +16	0/14	0

treatment had been initiated. Encouraged by these findings, attempts were made to arrest the disease at later stages of its development by commencing the therapy at 8, 10, or 13 days after birth and by increasing the dosage of ALS per inoculation to 0.1 ml, or even to 0.2 ml. Only when the injections were initiated 8 days after birth, did a few (22%) of the subjects make a full recovery (Table III, Experiment 3), though in one group of animals (Table III, Experiment 5), in which initiation of therapy was delayed until the 10th day, a significant prolongation of life was observed in 50% of the animals.

The marked capacity of ALS to abort the development of runt disease in infant BN rats was unexpected, since pilot trials had revealed that the same

batch of ALS was only marginally effective in prolonging the survival of BN skin grafts on young adult (100–120g) Lewis hosts. Treatment of these animals with 2 ml of antiserum subcutaneously on days 0, 2, and 5 only prolonged the lives of their homografts by 2–3 days.

Comparison of the present results obtained with ALS (Table III) with those obtained with isoantiserum (see Fig. 1 and Table IV) indicates that the latter constitutes the more effective protective agent in terms of the age of the hosts at which it is still effective.

DISCUSSION

The work described above and summarized in Table IV indicates that, in rats, the capacity of neonatally inoculated adult lymphoid cells to cause runt or homologous disease in homologous hosts can be mitigated or abolished by inoculating the latter with: (*a*) lymphoid cells from normal or specifically sensitized isologous adult donors; (*b*) isoantiserum specifically directed against a major alien histocompatibility antigen present in the attacking cells and absent in the host; or (*c*) rabbit anti-rat lymphocyte serum (ALS). These findings corroborate and extend previous observations on the therapy of runt disease in mice by means of isologous lymphoid cells or isoantisera (11–16), and in rats by isoantisera (22, 23).

The timing of the administration of lymphoid cells from normal isologous donors was found to be extremely critical. Only when the harmful and protective inocula were mixed before inoculation or, if administered separately, were given almost simultaneously via different veins was protection conferred. When both inocula were given intravenously, if the protective inoculum either preceded or followed the harmful one by as little as 4 hr, the protection obtained was greatly reduced. Likewise, if both inocula were injected at the same time, but by different routes, the protection afforded was much less than when both were injected intravenously. These observations sustain the thesis that a necessary condition for the conferral of protection by unsensitized isologous lymphoid cells is that they must be able to establish a prompt and initimate confrontation with the homologous target cells. It is possible that some of the cellular events which result from this confrontation in vivo are similar to those which occur when lymphocytes from two individuals are mixed and cultured in vitro—i.e., mixed lymphocyte interactions (30). If this is the case, then the antigen-sensitive cells which are presumed to be responsible for these reactions might either transform directly into effector cells, or might induce precursor cells present in the same population to differentiate into such cells.

Even when equivalent numbers of lymphoid cells from the homologous and isologous adult donors are introduced, and assuming that the proportions of specific antigen-reactive or immunologically competent cells present in each population are similar, the isologous host type "protective" cells are at a con-

siderable disadvantage. At all sites in the host the homologous cells are likely to undergo intimate exposure to host cellular antigens, so that every cell present gets an opportunity to produce a clone of effector cells. However, the chances will be much less that all the competent cells in the isologous cell population will make the necessary contacts with homologous cells for stimulation to produce effector clones.

In the light of this reasoning, it is perhaps surprising that the isologous cell inocula were able to exert any perceptible protective effect at all when administered independently from the homologous cells. Indeed, their impact might well have been much greater had it been possible to introduce them into the rat hosts several days before inoculation of the attacking cells, allowing them more time to become thoroughly assimilated and functionally efficient.

The superior protective properties of isologous lymphoid cells from sensitized donors—these were still effective when administered 3 or 4 days after the attacking cells—is probably a function of the high proportion of specific effector cells included. In the light of Wilson's (31) in vitro studies, as many as 1% of the inoculated isologous cells may have been capable of destroying homologous donor cells with which they established intimate contact.

The capacity to abort the development of runt disease in infant rats affords a very effective means of differentiating between lymphoid cell populations derived from normal and from specifically sensitized isologous donors, respectively. It had previously been shown, in both mice and rats, that one can also differentiate between normal lymphoid cells and those from specifically sensitized donors on the basis of their capacity to cause runt disease, or various parameters of this syndrome, such as splenomegaly, on inoculation into infant homologous hosts (4, 11, 13). However, in the rat, local expressions of GVH reactivity, such as the lesions produced at the sites of inoculation of parental strain donors cells beneath the renal capsule (32)[1] or in the skins (lymphocyte transfer reactions [33, 34]) of genetically appropriate F_1 hybrid hosts, afford a much less satisfactory means of distinguishing between normal and sensitized "attacking" lymphoid cell populations.

Compared with the protection afforded by cells from even sensitized isologous donors, that conferred by repeated injections of isoantiserum was truly remarkable because of its capacity to arrest runt disease at a relatively late stage of its development. Therapy initiated as late as the 10th or even 13th day was frequently effective. Presumably the effectiveness of this agent was a function of its specific, complement-mediated cytotoxic effect on the homologous lymphoid cell population (35). Obviously, as immunologic effectors, antibody molecules should afford a more effective means of eradicating or inactivating a chimeric cell population than some kind of cell-bound effector mechanism.

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[1] Elkins, W. L. Personal communication.

That complete recovery can result from late treatment of rats with isoantiserum is important, since it shows that runt disease is not the inevitable sequel to irreparable damage inflicted immunologically upon the host during the first few days of the GVH reaction (1). It also implies that, in the context of the present experiments, some kind of immunological attack must still be taking place against the hosts as late as 13 days after inoculation of the Lewis cells.

Jaffe (36) has recently studied the treatment of homologous disease in chick embryos, using inhibition of splenomegaly as a parameter of the therapeutic effect of inocula of normal or sensitized leukocytes (present in blood), or antiserum from isologous adult donors. Normal isologous cells only conferred protection if administered 3 days before the putatively harmful homologous lymphoid cell inoculum, whereas cells or antiserum from sensitized donors both afforded significant degrees of protection when administered at the same time as the attacking cells. Jaffe considers that pretreatment with the unsensitized cells was necessary to enable them to become established and furnish the embryo with a partially functional immunologic apparatus.

Although it has been well-established that pretreatment of an adult donor with heterologous anti-lymphocyte serum (26–28) will impair or abolish the capacity of its lymphoid cells to cause runt disease on inoculation into genetically appropriate hosts, we are not aware of any reports of ALS treatment of infant hosts destined to develop the disease. In the present study, it was found that repeated injections of rabbit anti-rat thymus serum saved the lives of rats inoculated with it, as late as the 6th or even the 8th day after receiving Lewis lymphoid cells. However, it was less effective than isoantiserum.

The finding that some BN rats recovered from runt disease after receiving initial inoculation of ALS at 6–8 days suggests that in this experimental situation the action of this agent may be directed against the efferent rather than the afferent limb of the immunological reflex.

It is important to point out that any analysis of the mechanism(s) underlying the protective action of the various agents described must take into account the demonstration by McBride et al. (37) that GVH reactions in chick embryos can induce a precocious maturation of immunologic competence on the part of host cells.

Although all the survivors of the various experiments described were challenged with skin grafts from Lewis donors to determine whether their early exposure to Lewis lymphoid cells had affected their subsequent capacity to react against Lewis tissue antigens, none was found to manifest the slightest degree of tolerance. This observation is not surprising in view of the fact that with the rat strain combination employed lymphoid cells (unlike bone marrow cells) are very ineffective in inducing tolerance of skin homografts. The findings do not exclude the possibility that some of the animals tested may have been lymphoid cell chimeras (4).

SUMMARY

Using rats of the Lewis and BN (Ag-B locus incompatible) isogenic strains, a comparative study has been made of the capacity to prevent or mitigate the development of runt disease with: (*a*) lymph node cell suspensions from normal adult BN rats, (*b*) node cells, or (*c*) serum from donors sensitized against Lewis tissue antigens, or (*d*) heterologous anti-lymphocyte serum (ALS) raised in rabbits against rat thymocytes.

Following a standard intravenous or intraperitoneal inoculation of 20×10^6 Lewis node cells into neonatal BN hosts, there are cutaneous manifestations of runt disease within 11–15 days and death invariably takes place within 20 days. However, complete protection is afforded by administration of a similar number of normal BN node cells via a different vein, or admixed with the otherwise harmful Lewis node cells. However, timing of the administration was crucially important—precedence or delay by as little as 4 hr resulted in a great impairment of protection. When the inoculations of the two cell suspensions were separated by 24 hr, no protection was afforded. These and other observations suggested that a necessary condition for protection of the hosts by unsensitized isologous cells requires that they establish a prompt and intimate confrontation with the homologous target cells.

At the same dosage level, suspensions of node cells from sensitized isologous donors were much more effective therapeutically, saving the lives of 92% of treated subjects when administered after a delay of 3 days, and of 19% when the delay was 4 or 5 days.

Of the various immunotherapeutic agents studied, daily injections of 0.2 ml of isoantiserum gave the best results, and could totally reverse the course of the disease even when initiated at age 10–13 days and subjects already presented symptoms. ALS, although inferior to isoantiserum at the dosage levels tested, proved to be superior to sensitized isologous cells as a protective agent, since the initiation of daily injections after delays of 6 or 8 days were still effective.

The observations that delayed treatments of infant rats with isoantisera or ALS resulted in complete recoveries sustain the thesis that the lesions responsible for the fatal outcome of runt diseases are not inflicted at a very early stage. The efficacy of both isoantisera and ALS as a means of inhibiting the progression of homologous disease also suggests that they may have therapeutic value in situations where this condition is encountered.

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