THE EFFECTOR CELLS IN *IN VITRO* SEMI-SYNGENEIC CYTOTOXICITY AND THEIR FUNCTIONS IN THE *IN VIVO* PHENOMENON OF THE GRAFT-VERSUS-HOST REACTION

By

KENNETH K.Y.FUNG, B.Sc.,M.Sc.,M.D.

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Department of Immunology
Faculty of Medicine
University of Manitoba
Winnipeg Manitoba
Canada

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ABBREVIATIONS

GVH = Graft-versus-host

PEC = Peritoneal exudate cell

PLN = Peripheral lymph node

PMN = Polymorphonuclear

^{51}Cr = Chromium 51

MHC = Major Histocompatibility Complex

Mls = M - Locus

MLR = Mixed leukocyte reaction

H-2 = Histocompatibility - 2 locus

Hh = Hybrid Histocompatibility (Hemopoietic Histocompatibility)

Ir = Immune response gene

CMC = Cell-mediated-cytotoxicity

PFC = Plaque-forming-cell

SRBC = Sheep red blood cell

LATS = Long Acting Thyroid Stimulator

LT = Lymphotoxin

LAD = Lymphocyte Activating Determinant

LAF = Lymphocyte Activating Factor

ITL = Initiator T lymphocyte

RTL = Recruited T lymphocyte

MIF = Migration Inhibitory Factor

AEF = Allogeneic Effect Factor

MF = Mitogenic Factor

CPM = Counts Per Minute

RS = Recognition Structure

RS_A = Recognition structure for the A antigen

RS_B = Recognition structure for the B antigen

Anti-RS = Anti-recognition Structure

RPMI = Rosewell Park Memorial Institute

HBSS = Hank's Balanced Salt Solution

BBS = Borate Buffered Saline

DS = Dulbecco's Solution

PPD = Purified Protein Derivative

EC = Effector Cell

TC = Target Cell

Semi-syngeneic = Semi-allogeneic
e.g., parental A/A cells are semi-syngeneic or semi-allogeneic to the (A x B) F_1 cells

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ABSTRACT

Graft-versus-host reaction was induced in adult F_1 hybrid mice with the transplantation of one of the two strains of parental spleen cells. The immunocompetent cells from these GVH-induced F_1 hosts showed a semi-syngeneic cytotoxicity or F_1 anti-parent immune reaction which was quantitated by the *in vitro* assays of the lysis of ^{51}Cr-labelled target cells. The route of induction of the GVH reaction was important in determining the degree of semi-syngeneic cytotoxicity, and the peritoneal exudate cells were observed to be most effective in eliciting the *in vitro* F_1 anti-parent immune response.

Investigations on the mechanical aspect of the semi-syngeneic cytotoxicity reaction revealed that the GVH reaction appeared to activate the F_1 macrophages to become cytotoxic effector cells since they were shown to possess surface adherence and were exclusively sensitive to silica particles. Non-adherent F_1 immunocompetent cells were found incapable of initiating the semi-syngeneic cytotoxicity reaction. Moreover, irradiation of the F_1 effector cells abrogated the semi-syngeneic cytotoxicity reaction.

The semi-syngeneic cytotoxicity reaction, mediated by the GVH-induced F_1 macrophages, could be enhanced by both *in vivo* and *in vitro* addition of normal syngeneic F_1 amcrophages. The adoptive transfer of GVH-induced F_1 PECs into other syngeneic F_1 recipients undergoing GVH reactions resulted in an increased semi-syngeneic cytotoxicity response as measured by the CMC assays, and also a decrease of the *in vivo* GVH reaction as evidenced by ; (1) decreased spleen indices relative to those

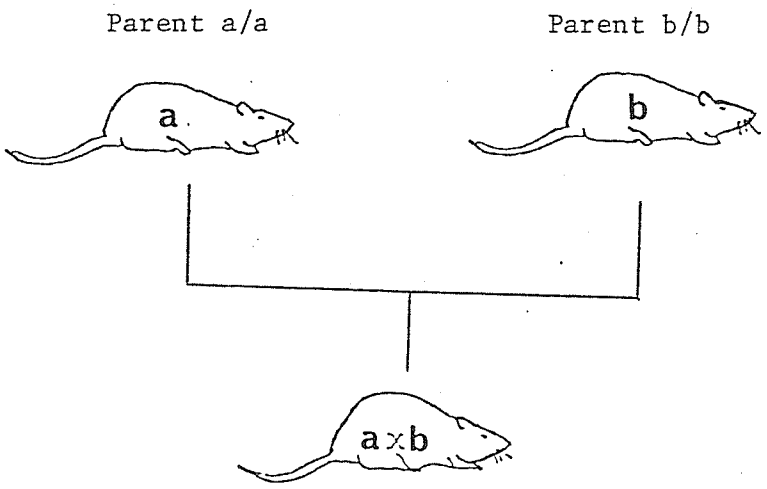
GVH-induced F_1 animals without receiving additional GVH-activated F_1 PECs, and (2) increased survival rates of lethally irradiated and GVH-induced F_1 recipients relative to those without receiving additional GVH-activated PECs. Such capacity to suppress an in vivo GVH reaction by the GVH-activated F_1 cells could be abrogated by irradiation. Moreover, certain degree of specificity seems to exist in the in vivo GVH reaction suppression. This type of speciificity is reflected by the in vitro observation in the preferential target cell lysis experiments. The GVH activated F_1 immunocompetent cells were shown to mediate the host-versus-graft reaction in producing the spontaneous resolution of the in vivo GVH reaction.

The underlying mechanism of the semi-syngeneic cytotoxicity reaction was explored. Investigations on the immunological aspect of the mechanism revealed that, during a GVH reaction, the F_1 host immunocompetent cells exhibited a preferential cytotoxic effect on the parental H-2 genotype target cells; i.e., when the H-2 genotype of the target cells used in CMC assays and the H-2 genotype of the parental cells used in GVH induction were identical, the lysis of target cells was significantly higher than the situation in which the H-2 genotype of the target cells and the parental cells were different. In addition, the F_1 immunocompetent cells were found capable of reacting against the histocompatibility antigens of the parental cells, demonstrating the mediation of the semi-syngeneic cytotoxicity or F_1 anti-parent reaction via the antigenic determinants of the H-2 complex.

INTRODUCTION

A graft-versus-host reaction results from the recognition of host tissue-antigens which do not exist in the transplanted immunocompetent donor cells. Circumstances initiating GVH reactions include situations in which the host will accept a graft without the capacity of rejection. The principle of GVH reaction is classically illustrated by the condition that F_1 hybrid animals will accept immunocompetent cells from either parental strains, but the grafted cells from one parental strain are confronted in the tissues of the F_1 host with antigens inherited from the other parental strain. The grafted cells proceed to attack the F_1 host tissues bearing such foreign antigenic determinants resulting in the experimental form of graft-versus-host reaction (Oliner et al 1961). The principle of such a unidirectional reaction is illustrated in Figure 1.

There are basically two categories of GVH reaction; systemic and localized. The immunologists have done considerable work on the systemic GVH reactions, while the pathologists are more interested in studying the localized GVH reactions. Experimental evidence of systemic GVH reaction was initially provided by the production of "runting diseases" in newborn mice which were injected with adult lymphoid cells (Simonsen,1957). Apart from this type of classical hybrid wasting disease, other types of systemic GVH reactions have been described. For example, adult mice previously made tolerant to another strain by neonatal inoculation with immunoincompetent cells from the other strain, were noted to develop "runting diseases" when they were grafted with immunocompetent cells from



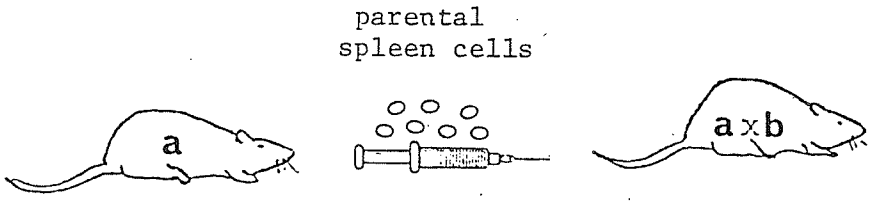


Figure 1 - Principle of Experimental GVH Reaction

the donor strain origionally used in the induction of neonatal tolerance (Billingham et al 1955; Billingham and Silvers,1961). In the case where the parabiotic surgical union of an animal (graft) with an immunologically non-responsive partner (host) through vascular anastomoses, the syndrome of parabiosis intoxication characterized by severe anemia, weight loss, and death of the non-responsive partner (host), had been identified to be one form of systemic GVH reactions (Eichwald et al,1959). Lethally irradiated animals, when transplanted with allogeneic bone marrow cells, usually recovered from the primary effect of irradiation, but they eventually developed "secondary diseases" due to the activities of the immunocompetent cells in the transplanted bone marrow inoculum (Trentin,1956).

Besides the systemic category, several localized forms of GVH reactions have been described. These included the intrarenal GVH reactions produced by inoculating parental lymphoid cells into the renal capsules of F_1 hosts (Elkins,1964), and the intracutaneous GVH reaction observed in hamsters and guinea pigs when parental spleen cells were injected intradermally (Brent and Medarwa,1966). When adult chicken leukocytes were distributed over the chorioallantoic membrane of a genetically unrelated embryo, white focal "pocks" developed. Using this type of local GVH reaction, the small lymphocytes were established as participants in such reactions (Simonsen,1967). Popliteal lymph node (PLN) hypertrophy, or enlargement, after the injection of parental lymphoid cells into the foot-pads of animals such as hamsters and rats, has also been used to quantitate local GVH reactivities (Grebe and Streilein,1974).

The importance of GVH reaction in immunology is mainly two-fold. From an academic point of view, GVH reaction has been and will

continue to be a useful experimental approach in studying immunobiology, the immune response, and immunoregulation. In clinical investigations, GVH reactions have been important in situations of : intrauterine blood transfusion of Rh erythroblastosis fetalis (Naiman,1969), the transfusion of blood into congenitally immune deficient infants (Hathaway,1965), and bone marrow transplantation studies (Kretschner,1970). GVH reaction today remains the major stumbling block in the success of bone marrow tranplants. Approximately 70% of patients receiving bone marrow grafts develop GVH reactions (Thomas et al,1975); and about half of these die from GVH related infections, or from the GVH reaction itself (Bunn,1977; Rosen et al,1978). Granulocyte transplantations have recently been used with some success in acute leukemic patients (Graw et al,1970), but GVH reaction is significantly induced with platelet transfusions (Mathe et al,1974), presumably due to the simultaneous transfer of contaminating leokocytes (Cohen et al,1979).

Because of academic significance and extensive clinical applications, many research efforts have been devoted to study the phenomenon of graft-versus-host reaction. In the following review of literature, relevant significant findings are described.

REVIEW OF LITERATURE

An enormous amount of literature is available on the various aspects of the GVH reaction, and since the review of literature is not meant to be all-inclusive, only articles pertinent to the theme of the present thesis are presented. The review is divided into three main sections : Sequelae of graft-versus-host reactions, Immunology of GVH reactions, and Immunology of host-versus-graft (HVG) reactions. Within each section, significant observations will be described.

SEQUELAE OF GRAFT-VERSUS-HOST REACTION

In the animal model, graft-versus-host reactions can be induced by the injection of parental lymphoid cells into the appropriate F_1 hybrids, and in applying this technique to newborn mice, the GVH syndrome observed is historically known as "runting diseases". In clinical situations, GVH diseases in humans are usually the result of bone marrow transplantations. In the animal model, GVH reactions have been extensively investigated; its manifestations and sequelae of the phenomenon are described in the following.

Manifestations of Graft-versus-host Reaction

When parental lymphoid cells are injected intravenously or intraperitoneally into the appropriate F_1 hybrids, systemic GVH reactions can take either an acute or a chronic course, depending on the number of grafted parental cells, the age of the hybrid at the time of transplantation, and the extent of histoincompatibility involved (Brent and Medawar,1966).

In the animal model, anatomical manifestations of the GVH syndrome as initially described in "runting diseases" include the failure of normal somatic development of the newborn, splenomegaly, hepatomegaly,

hypertrophy, then atrophy of the lymphoid organs, and lesions of the cutaneous tissues (Billingham,1968). The hallmark of the systemic process is significant hyperplasia of the splenic parenchyma. Early splenic enlargement had been attributed to the proliferation of the grafted donor cells, but in later periods, splenomegaly is mainly due to the proliferation of the host lymphoid cells (Auerbach and Globerson,1966; Hilgard,1970; Bonney and Feldbush,1973; Bennett and Hand,1978).

Histologically, the follicular structure of the spleen becomes obliterated and the majority of the organ is occupied by blast-like cells and necrotic foci (Simonsen,1957). This acute stage of lymphoid organomegalies is followed by a stage of pronounced hypoplasia, particularily of the thymus. The most important physiological changes are immunological, hematological, and hepatic dysfunctions. Decreased immune responsiveness results in increased susceptibility to bacterial and viral infections (Elkins,1971). In many murine strain combinations tested, hosts undergoing GVH reactions developed remarkably frequent, malignant reticuloendothelial tumors (McBride,1966).

The manifestations of GVH reactions in the human patients studied are usually secondary to bone marrow transplantations. The signs and symptoms of GVH disease appear from 10 to 30 days after grafting of the bone marrow. The earlier the manifestations appear, the more serious the reaction and prognosis (Cline et al,1975). The clinical picture in these patients consists of dermatitis with erythematous maculopapular eruptions spreading all over the body, hepatitis accompanied by jaundice, elevated hepatic enzymes, and gastrointestinal disturbances (Thomas et al, 1975). These synptoms will usually lead to generalized immunodeficiency

states, complicated by severe infections, resulting in septic shock and death (Wells and Ries,1978).

Experimental GVH reactions in the animal model do not, as a rule, cause the death of the host. In fact, if the host can survive the initial GVH syndrome, spontaneous recovery is expected, and a type of secondary GVH reaction is not easily inducible, if not impossible. This type of natural resolution of GVH reaction manifestations observed in experimental animals unfortunately does not occur in humans because the simultaneous presence of intrinsic diseases in humans are absent in the experimental animals.

Remission of Graft-versus-host Reaction

If the dogma of transplantation immunology is correct in asserting that the F_1 hybrid immunocompetent cells are incapable of reacting against the parental lymphoid cells, it seems possible that when a single parental immunocompetent cell, injected into the F_1 host, and given sufficient period of incubation, would proliferate and produce a full blown picture of GVH syndrome; but this is not the case. In fact, many studies have shown that F_1 animals which survived the acute stage of GVH reaction usually recover (Gowans,1962).

Natural remissions of both systemic and localized GVH reactions in experimental animals have been extensively reported. When lethally irradiated (CB x MHA) F_1 hamsters were inoculated with parental MHA lymphoid cells, the severe cutaneous reaction of lethal epidermolysis (one form of systemic GVH reactions) were produced. But using normal (CB x MHA) F_1 hmasters as recipients of intravenously inoculated parental MHA lymphoid cells, the severe cutaneous reaction observed in lethally

irradiated syngeneic F_1 hamsters was absent, and the F_1 hosts eventually returned to a normal state with no apparent pathological sequelae (Streilein and Billingham,1970a).

Studies on localized GVH reactions also show that the pathological manifestations resolve spontaneously. By injecting parental lymphoid cells into the renal subcapsular spaces of the F_1 recipients to demonstrate local GVH pathology, it was observed that after the initial reaction, diminishing inflammatory infiltrate appeared by the 14th day. At the end of the 40th day, there was little macroscopic evidence that GVH reaction had occured (Elkins,1964). Using (DA x Lewis) F_1 rats as hosts, parental Lewis lymphoid cells were inoculated intradermally to initiate a localized GVH reaction. After the initial skin manifestations, the reaction regressed to a stage where barely perceptible residual lesion, identified by a necrotic skin nodule remained on the F_1 hosts (Streilein and Billingham,1967). By injecting parental lymphoid cells into the foot-pads of the appropriate F_1 hybrids, local GVH reactions, quantitated by assaying popliteal lymph node weights consistently followed a self-limited course (Grebe and Streilein,1974).

The self-limited nature of GVH reaction has also been quantitated by delayed hypersensitivity reactions involving the measurement of the thickness of the foot-pads of F_1 hosts injected with parental lymphoid cells. When parental C57BL/Rij spleen cells were transplanted into (C57BL/Rij x CBC/Rij) F_1 mice, GVH reactivities were noted to be maximal from day 5 to day 8, and thereafter the reactivities decreased progressively (Wolters and Benner,1978).

Spontaneous remissions of GVH reactions seem to involve

certain immunoregulatory mechanisms. Features of the GVH syndrome usually subside in experimental animals when the lymphoid organs are replenished by proliferating host lymphoid cells (Fox,1966). In the characteristic development of splenomegaly during GVH reactions, the weights of the spleens, after reaching peak values, decline progressively. This is followed by complete recovery (Simonsen and Jensen,1959). It has been suggested that the host spleen provides an immunoregulatory microenviroment in which cell-mediated immune response, including GVH reaction, are modulated or regulated (Grebe and Streilein,1976).

Absence of Secondary Graft-versus-host Reaction

In addition to the spontaneous remission of GVH reaction in the genetically tolerant F_1 hybrid host, certain mechanisms seem to produce in the host animal a state of refractoriness to subsequent GVH-inducing challenges, and no secondary GVH reaction parallel to the secondary antibody response can be initiated. This phenomenon was first made in experiments in which adult (CBA x C57BL/6) F_1 mice, previously injected with parental C57BL/6 spleen cells, failed to develop GVH reaction on subsequent injection of either strain of parental spleen cells (Fox and Howard,1963). The observation was later supported by the fact that rats which survived the first GVH reaction were subsequently found to be staunchly resistant to a second inoculation of lymphoid cells from the same parental strain (Field and Gibb,1966). Such resistance to a secondary challenge has also been demonstrated in parabiosis studies during the acute stage of the GVH syndrome where certain unidentified humoral factors were suggested to be responsible for the refractory state (Field and Cauchi,1967). In the case of F_1 hybrid hamsters that have

survived the early phase of GVH disease, a subsequent challenge with lymphoid cells of the original donor genotype resulted in similar refractoriness as reported in other animals tested (Streilein,1972).

The absence of a secondary GVH reaction has been studied in its specificity, and both "specific refractoriness" as well as "non-specific refractoriness" have been identified. Using DA, Fischer (parental donors) and (DA x Fischer) F_1 hybrid rats (GVH recipients) as an example, the term "specific refractoriness" referred to a situation in which the parental strain used in both the primary (e.g.,DA) and secondary (e.g.,DA) challenges were identical, while "non-specific refractoriness" indicated the situation where the parental strain used in the primary (e.g.,DA) and the secondary (e.g.,Fischer) challenges were dissimilar. In both situations, the absence of a secondary GVH reaction was confirmed (Grebe and Streilein,1976).

These observations implicated a situation in which, parental immunocompetent cells, after exposure to semi-allogeneic F_1 host tissues, induced certain immunoregulatory mechanisms in the F_1 hybrids so that a secondary GVH-response is modulated or suppressed.

Failure of Adoptive Passage of Graft-versus-host Reaction

If it is true that the F_1 generation is genetically tolerant to parental immunological challenge, then it should be possible to produce the GVH reaction serially from the primary host to a syngeneic secondary host by adoptive transfer of lymphoid cells. It was however, impossible to elicit "adoptive runting diseases" in mice by transferring spleen cells from the first F_1 recipient to the second syngeneic recipient (Russel,1961). Moreover, evidence has been presented that within 24 hours after injection into the newborn F_1 hybrids, parental lymphoid cells lost their capacity to

initiate GVH reaction in a secondary host (Simonsen and Jensen,1959). Using isogeneic rodents and inbred chicken strains, a few studies have only been able to achieve at best, one or two passages before the cell suspensions lost their capacity to incite GVH reactions (Ramseier and Billingham,1966; Steinmuller,1967).

Attempts to passage the GVH reaction serially in hamsters beyond the tertiary host by adoptive cellular transfer were unsuccessful (Streilein and Billingham,1970a). Using adult (Fischer x DA) F_1 rats as primary and secondary hosts in serial passage experiments, it was noted that there was only popliteal lymph node enlargement (local GVH) in the secondary host and none in the tertiary host (Grebe and Streilein,1976).

The failure to transfer serially a GVH reaction in syngeneic hosts can be explained by two possibilities. The first possibility is that the donor lymphoid cells were being serially diluted to such a level as to be ineffective in inducing GVH reaction when transferred to the secondary or tertiary hosts. The possibility of such a diluting effect on the donor cells seems unlikely because there is inadequate explanation to account for the inability of donor cells to proliferate so as to compensate for the diluting effect in a genetically tolerant host.

The second possibility is that certain immunoregulatory mechanisms take place during a GVH reaction within the primary host, thus rendering the donor cells incapable of initiating the reaction in the syngeneic secondary host. Evidence supporting this assumption has been reported in studies using T_6 chromosome markers in donor cells. It was observed that during GVH reaction, parental donor cells in active mitosis constituted only about 1% in the host spleen by the 14th day, indicating