THE Qa-1 ANTIGENIC SYSTEM

Relation of Qa-1 Phenotypes to Lymphocyte Sets,

Mitogen Responses, and Immune Functions*

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The discovery of the TL system of thymocyte-surface antigens, and the finding that the *Tla* locus is situated next to *H-2D*, were followed by the development of the congenic strains B6-*Tla^a* and A-*Tla^b* derived from recombinants between *H-2D* and *Tla* (1). These recombinant congenic strains served to isolate the *Tla* region from the major histocompatibility complex, commonly defined as the region from *H-2K* to *H-2D*. Thereafter the usual antiserum used for TL typing of thymocytes and leukemias was prepared by immunizing (B6 × A-*Tla^b*) TL⁻ hybrids with A strain cells (genotype *Tla^a*, phenotype TL.1,2,3). Subsequently this antiserum was found to react with peripheral lymphocytes, and these reactions proved to be distinct from TL (2), as expected from the restriction of TL antigens to thymocytes. The antigenic system defined by the reactions of (B6 × A-*Tla^b*) α-A-strain serum with peripheral lymphocytes is referred to as the Qa-1 system, and the antiserum when used in this context is called α-Qa-1. We report here the Qa-1 phenotypes of peripheral T cells according to cytotoxicity assays, mitogen responses, and the effects of excluding Qa-1⁺ cells from assays of immune function in vitro.

We do not yet know how complex the Qa-1 system may be. Thus when two or more cell sets are denoted Qa-1⁺ on the basis of their reactions with Qa-1 antiserum, it is not implied that they necessarily express the same Qa-1 antigen. The Qa-1 system may comprise more than one cell-surface component, determined by different genes in the region spanning the *Tla* locus.

Materials and Methods

Animals. Except for C57BL/6J (B6), obtained from The Jackson Laboratory, Bar Harbor, Maine, all mice were bred at Memorial Sloan-Kettering Cancer Center or the Dana Cancer Center, and were used at 6- to 12-wk of age.

Antisera. The antiserum (B6 × A-*Tla^b*) anti-A strain leukemia ASL1, called α-TL.1,2,3 in reference to its reactions with thymocytes, is referred to in the text as α-Qa-1 in reference to its reactions with peripheral T cells (Introduction). The α-Thy-1.2 serum was prepared by

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immunization of Thy-1 congenic mice. The preparation and use of Ly antisera are described elsewhere (3, 4).

Complement. This study was conducted with pools of rabbit serum that had been rigorously screened and selected for high complement levels combined with low inherent cytotoxicity, usually four to five rabbits out of 30 were chosen, as described in reference (2) which also gives details of cytotoxicity assays for Qa-1 and the calculation of cytotoxicity indices.

Elimination of Cell Sets with Antibody and Complement. 20-30 × 10^6 cells/ml were incubated with α-Qa-1 (1:25), α-Thy-1.2 (1:40), or NMS (1:50), in either RPMI-1640 or medium 199 for 20 min at 4°C. After washing once, the cells were resuspended at 20-30 × 10^6/ml in rabbit serum diluted 1:20 (complement) and incubated at 37°C for 30 min. Before further use, the cells were washed twice in medium plus 5% fetal calf serum (FCS).[1] The percentage of dead cells was determined by trypan blue exclusion.

Mitogen Activation. For assay of mitogen responsiveness, spleen cell suspensions pretreated with antiserum plus complement (C) were adjusted to 4 × 10^6 viable cells/ml and 0.1 ml was cultured with 0.05 ml of either 2 μg/ml concanavalin A (Con A), Sigma Chemical Co., St. Louis, Mo., 50 μg/ml lipopolysaccharide (LPS), Difco Laboratories, or phytohemagglutinin M (PHA-M) (1:64; Difco Laboratories) in RPMI-1640 containing 10% FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Quadruplicate cultures were incubated in 16-mm round bottom wells (Linbro Chemical Co., Hamden, Conn.) for 48 h, at which time 2 μCi [^{3}H]thymidine was added. After an additional 18 h, the cultures were terminated and [^{3}H]thymidine incorporation assessed.

Antibody Responses In Vitro

PRIMARY RESPONSE. After treatment with α-Qa-1 (+C) or normal mouse serum (NMS) (+C), 10 × 10^6 viable spleen cells were incubated for 5 days with 3 × 10^6 sheep erythrocytes (SRBC) (Colorado Serum Co., Denver, Colo.) according to Mishell and Dutton (5). In some cases, a modification of this procedure was used, allowing efficient stimulation of 5 × 10^6 cells (6). Duplicate cultures were harvested on days 4 and 5 and assayed for direct plaque-forming cells by a slide modification of the Jerne plaque assay (6).

SECONDARY RESPONSE. Mice were primed by intraperitoneal injection of 2 × 10^8 SRBC and pertussis vaccine containing 3 × 10^9 bacteria (Eli Lilly and Co. Indianapolis, Ind). 8 Days later, spleen cell suspensions were treated with either α-Qa-1 (+C) or NMS (+C) and 4 × 10^6 remaining viable cells were incubated for 5 days with 3 × 10^6 SRBC. Duplicate cultures were harvested on days 3, 4, and 5, and assayed for direct and indirect plaque-forming cells (PFC) as described above. Peak responses were obtained on day 4.

Generation of Suppressive Cells. Two methods were used: (a) SRBC-stimulated suppressor cells were prepared according to Eardley and Gershon (7). Briefly, 3.5 × 10^7 spleen cells from B6-Tla^a (Qa-1$^+$) mice or B6 (Qa-1$^-$) mice were incubated for 3 days with 12 × 10^6 SRBC in 3.5 ml of culture medium in the absence of 2-mercaptoethanol; (b) Con A-activated suppressor cells were prepared as described previously (6, 8). Briefly, 3-4 × 10^7 spleen cells from Qa-1$^+$ mice were cultured with 2 μg/ml Con A at a concentration of 3-4 × 10^6 cells/ml in RPMI-1640 containing 10% FCS, and 5 × 10^{-5} M 2-mercaptoethanol.

Mixed Lymphocyte Cultures (MLC). Spleen cells were cultured in RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 5 × 10^{-5} M 2-mercaptoethanol, and 15% heat-inactivated FCS. Responding and X-irradiated stimulating cells (1,200 rads) were cultured at a 1:1 ratio in 20-ml vol at a concentration of 2 × 10^6 cells/ml of each type. Proliferation was measured on days 1-5 by uptake of [^{3}H]thymidine. Triplicate 200-μl aliquots were removed and cultured in microtiter plates with 2 μCi [^{3}H]thymidine for 6-8 h. Samples were harvested onto glass fiber filters, and [^{3}H]thymidine incorporation was determined by liquid scintillation spectrometry.

Cell-mediated cytotoxicity (CMC) was measured on day 5 by a 4-h ^{51}Cr release assay. Con

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[1] *Abbreviations used in this paper:* C, complement; CMC, cell-mediated cytotoxicity; Con A, concanavalin A; CTL, cytotoxic lymphocyte; FCS, fetal calf serum; LNC, lymph node cell; LPS, lipopolysaccharide with *Escherichia coli* 0127: B8; MHC, major histocompatibility complex: MLC, mixed lymphocyte culture; NMS, normal mouse serum; PFC, plaque-forming cell; PHA-M, phytohemagglutinin M; SRBC, sheep erythrocytes; Ts, T-suppressive activity.
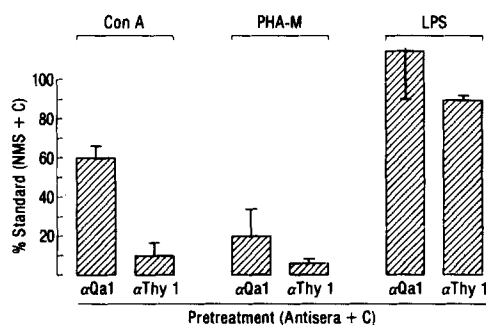
Fig. 1. Qa-1 phenotypes of cells involved in the proliferative response of B6-*Tla*^{a} spleen cells to the mitogens Con A, PHA-M, and LPS. Mean data ± SD for four tests. Controls not shown: B6 spleen cells (Qa-1⁻) preselected with α-Qa-1 responded similarly to B6 spleen cells preselected with NMS + C; B6 spleen cells preselected with α-Thy-1.2 + C gave results similar to those shown for α-Thy-1.2 + C in this figure.

A (Difco Laboratories) stimulated BALB/c (H-2^d) spleen cells (72 h with 4 μg/ml Con A at a concentration of 4 × 10^6 cells/ml or P815 mastocytoma cells (H-2^d) labeled with ^{51}Cr served as target cells. Lympholysis was measured in triplicate samples at target to effector ratios of 100:1–12.5:1. Percent CMC was determined by the formula:

$$\frac{\text{mean cpm experimental} - \text{mean cpm spontaneous release}}{\text{mean cpm maximum release} - \text{mean cpm spontaneous release}} \times 100.$$

Spontaneous release was determined by incubating target cells in medium alone; maximum release was determined by detergent lysis (5% Triton, New England Nuclear, Boston, Mass.) of target cells.

Distribution of Qa-1$^+$ Cells within the Lyt Sets. Whether or not each Lyt set contained Qa-1$^+$ cells was determined by the following procedure: Lyt and Qa-1 antisera were used in combination with each other (e.g., α-Qa-1 + α-Lyt-1.2 or α-Qa-1 + α-Lyt-2.2 or α-Lyt-1.2 + α-Lyt-2.2) in two-stage cytotoxic tests. α-Qa-1 was used at a final dilution of 1:50, α-Lyt-1.2 at 1:40, and α-Lyt-2.2 at 1:10. The Ly antisera were prepared as described previously (3, 4). To increase the proportion of Ly positive cells, lymph node cells were treated with α-Ig antisera (provided by Dr. U. Hammerling, Sloan-Kettering Institute) and complement as described (2) resulting in 70–80% Thy-1$^+$ cells. Cytotoxic indices in Table V are calculated as a percent of Thy-1$^+$ cells.

Results

Qa-1 Phenotypes of Cells Involved in Proliferative Responses to Mitogens In Vitro. For this purpose the proliferative responses of B6-*Tla*^{a} spleen cells preselected with α-Qa-1 plus C (the Qa-1⁻ population) were compared with the responses of equal numbers of unselected B6-*Tla*^{a} spleen cells (NMS + C). Fig. 1 shows that the Qa-1⁻ population has a distinctive profile of responses to the three mitogens Con A, PHA-M, and LPS, implying that α-Qa-1 defines a population of cells not identified by any other antigenic system. Evidently Qa-1$^+$ cells are not essential for response to LPS (unaffected by α-Qa-1 preselection) but are mainly responsible for the response to PHA-M (virtually abolished by α-Qa-1 preselection), either by proliferating themselves or possibly by inducing proliferation of Qa-1⁻ cells. Preselection with α-Qa-1 reduced the Con A response by 40%. This is in keeping with the broader reactivity of Con A as compared with PHA-M and suggests that the Con A responsive population includes both Qa-1$^+$ and Qa-1⁻ members. An indication that the Qa-1$^+$ cells which contribute
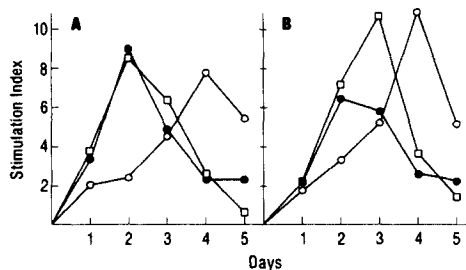
Fig. 2.   Uptake of [^{3}H]thymidine in MLC by equal numbers of B6-Tla^a spleen cells preselected by cytolysis with α-Qa-1 ($\square$), α-Thy-1 (O), or NMS ($\bullet$), and stimulated with irradiated AKR cells (panel A) or irradiated with BALB/c cells (panel B). This figure shows one of six experiments, with either B6-Tla^a or A/J responder cells (both Qa-1$^+$), which gave similar results. (Controls for Qa-1 specificity: in every test, control Qa-1$^-$ B6 or A-Tla^b responder cells pretreated with α-Qa-1 + C gave values indistinguishable from B6-Tla^a or A responder cells pretreated with NMS + C.)

to the Con A response do so directly by proliferating comes from other tests with lymph node cells (LNC). Before stimulation, roughly 30% of B6-Tla^a LNC are Qa-1$^+$; after activation by Con A, roughly 50% of the blast cells are Qa-1$^+$ (and 80% Thy-1$^+$).

Qa-1 Phenotypes of Cells Involved in Reaction to MHC-Incompatible Cells

PROLIFERATION (MLC).   Removal of Thy-1$^+$ cells markedly affected the tempo of the MLC response to irradiated MHC-incompatible stimulator cells, and their [^{3}H]-thymidine incorporation was substantially reduced at the time when control (NMS + C) responder cells showed maximal incorporation (48–72 h after initiation of culture). This suggested that elimination of a proportion of T cells could best be assessed in MLC assay by a delay in the tempo of the response. Fig. 2 shows that the tempo and degree of MLC activation in cultures lacking Qa-1$^+$ cells were virtually identical to those of cultures of control unselected responder cells (NMS + C). The experiment shown in Fig. 2 is one of six such experiments performed, all of which gave essentially similar results. Evidently Qa-1$^+$ cells need not be present for the proliferative MLC response to MHC-incompatible cells. In fact in one test elimination of Qa-1$^+$ cells enhanced incorporation during the 3rd day of stimulation.

Although Qa-1$^+$ cells are not necessary for proliferation, measured by MLC, they may nonetheless proliferate. We tested this by counting Qa-1$^+$ cells present after MLC with unselected spleen cells. (The stimulating cells were Qa-1$^-$ and therefore could not contribute to the count of Qa-1$^+$ cells.) After 72 h in culture, MLC-stimulated cell populations contained 41% Qa-1$^+$ cells and 51% Thy-1$^+$ cells, as compared with 20% Qa-1$^+$ cells and 30% Thy-1$^+$ cells in the unstimulated (control) populations. Thus during MLC either Qa-1$^+$ cells normally proliferate or the phenotype of some Qa-1$^-$ cells is converted to Qa-1$^+$.

CMC.   The following experiments were designed to indicate the Qa-1 phenotypes of cells responsible for the generation and effector function of cytotoxic lymphocytes (CTL). Representative data are shown in Table I: elimination of Thy-1$^+$ cells before MLC abolished cytotoxic activity of the stimulated population in subsequent CMC assays, but elimination of Qa-1$^+$ cells before MLC did not, indicating that Qa-1$^+$ cells are not required for the generation of CTL. Elimination of Thy-1$^+$ cells after MLC, immediately before CMC assay, greatly reduced lytic activity. CTL generated in MLC seem relatively resistant to α-Thy-1 + C, and complete elimination of cytolytic function was not achieved in all experiments despite two cycles of exposure to α-

TABLE I

Qa-1 Phenotypes of B6-Tla^a Cells Involved in the Generation and Effector Function of Cytotoxic Lymphocytes against MHC-Incompatible (BALB/c) Target Cells

Source of responder cells	Treatment of responder cells before MLR*	Treatment of responder cells after MLR*	Cytolytic activity 96 lysis % lysis (E:T‡ = 12.5:1)		
			Exp. 1	Exp. 2	Exp. 3
Spleen	NMS + C	—	59	24	20
	α-Qa-1 + C	—	56	21	27
	α-Thy-1 + C	—	<5	<5	<5
	—	NMS + C	71	76	55
	—	α-Qa-1 + C	74	51	58
	—	α-Thy-1 + C	40	24	4
			% lysis (E:T‡ = 10:1)		
			Exp. 1	Exp. 2	Exp. 3
Lymph nodes	NMS + C	—	19	10	28
	α-Qa-1 + C	—	46	25	67
	—	NMS + C	24		
	—	α-Qa-1 + C	29		

* After antiserum + C treatment, the numbers of remaining viable cells were equalized. MLR, mixed lymphocyte reaction.
‡ Effector-cell:target cell (ratio).

Thy-1 + C. Elimination of Qa-1$^+$ cells (two cycles of exposure to α-Qa-1 + C), after MLC, did not reduce CMC activity, indicating that CTL, in this experimental system, are Qa-1$^-$. In fact, these data indicate that Qa-1$^+$ LNC may exert an inhibitory effect upon the generation of CTL.

Thus major histocompatibility complex (MHC)-alloreactive Lyt-23 prekiller and killer effector cells are apparently Qa-1$^-$. No conclusions, however, should be drawn concerning the Qa-1 phenotype of Lyt-1$^+$ T$_H$ cells that amplify the response of Lyt-23 allogeneic prekiller cells because help and suppression are probably generated concomitantly (12), and thus a reduction in helper activity could be balanced by reduction of suppression.

Qa-1 Phenotypes of Cells Involved in the Production of Antibody to SRBC

INFLUENCE OF Qa-1$^+$ CELLS ON PRIMARY AND SECONDARY RESPONSES TO SRBC IN VITRO. Table II shows that the primary α-SRBC PFC response of the Qa-1$^-$ spleen population (selected with α-Qa-1 + C), on day 5 of culture, was more than six times that of equal numbers of the unselected population (NMS + C). The response of Qa-1$^-$ spleen cells from SRBC-primed mice, on day 4 of culture, was more than double that of the unselected population. These increments in PFC values for the Qa-1$^-$ population were sufficiently high to suggest that they might be due to elimination of suppression by the missing Qa-1$^+$ cells. This proposal was tested in the experiments reported below.

CONTRIBUTION OF Qa-1$^+$ CELLS TO T-SUPPRESSIVE ACTIVITY (TABLE III). (a) SRBC-induced suppressor activity: B6-*Tla^a* spleen cells were cultured with SRBC for 3 days to generate α-SRBC suppression and were then added in graded numbers, with

TABLE II

Participation of Qa-1$^+$ Cells in Primary and Secondary α-SRBC PFC Responses in Vitro

Antibody response to SRBC in vitro	B6-*Tla^a* spleen cells selected with:	PFC response (% of standard)	
		Day 4	Day 5
Primary*	NMS + C (standard)	(100)	(100)
	α-Thy-1 + C	5	4
	α-Qa-1 + C	235	640
Secondary‡	NMS + C (standard)	(100)	(100)
	α-Thy-1 + C	8	ND
	α-Qa-1 + C	225	ND

* Mean data for four experiments; the range of values for controls (NMS + C: standard) was 1,200–4,100 direct PFC per culture.

‡ Mean data for three experiments; the range of values for controls (NMS + C: standard) was 1,800–4,400 indirect PFC per culture.

TABLE III

Contribution of Qa-1$^+$ Cells to SRBC-Induced Suppression*

Treatment of spleen cells before stimulation by SRBC in vitro	Treatment of spleen cells after 3 days stimulation by SRBC in vitro	Cells added to fresh spleen cell cultures		
		2×10^5	5×10^4	5×10^3
		% reduction in PFC‡ (day 4)		
None	NMS + C	81 ± 4	52 ± 5	<10
None	α-Qa-1 + C	57 ± 6	<10	<10
α-Qa-1 + C	NMS + C	26 ± 5	<10	<10
None	α-Thy-1 + C	<10	<10	ND

* Mean data for four experiments in which the control values for 10^7 normal spleen cells plus SRBC (standard) without addition of educated cells averaged 8,410 ± 3,650.

‡ 100 − mean PFC count per culture/mean PFC count per control culture.

SRBC, to cultures of 10^7 unprimed B6 spleen cells. As indicated in Table III, elimination of Qa-1$^+$ cells before induction of anti-SRBC suppressor activity resulted in a substantial (>10-fold) reduction in the ability of the residual Qa-1$^-$ population to generate α-SRBC suppression. Elimination of Qa-1$^+$ cells after induction of α-SRBC suppressor activity also resulted in considerable (≈10-fold) reduction of T-suppressive activity. The Qa-1 specificity of elimination in this study was confirmed in control experiments in which suppression was not abrogated in parallel tests in which B6 (Qa-1$^-$) spleen cells were subjected to the same selection procedure with α-Qa-1 + C.

(*b*) Con A-induced suppressive activity: under certain conditions, inclusion of Con A in SRBC-stimulated spleen cell cultures results in a substantially reduced PFC response, and this is due to suppressive effects of Thy-1$^+$ cells (6, 8, 12). Such suppression was virtually eliminated if Qa-1$^+$ cells were removed from spleen cells before culture (Table IV; A). To determine whether Qa-1$^+$ cells were essential for both generation of T-suppressive (Ts) activity as well as for suppressor-effector activity, T cells (nylon wool purified) were selected with α-Qa-1 + C either before or

TABLE IV

Con A in Relation to Qa-1$^+$ Cells and Suppression

(A) Suppression of the primary in vitro SRBC PFC response by Con A: contribution of Qa-1$^+$ cells*

	% reduction	
T-Cell population	Exp. 1	Exp. 2
Unselected	98	96
Qa-1$^-$	39	0

(B) Contribution of Qa-1$^+$ cells to generation of Ts after Con A stimulation‡

Treatment of cells before in vitro stimulation by Con A	Treatment of cells after in vitro stimulation by Con A	Cells added to fresh SRBC-stimulated spleen cells	
		2 × 10^5	5 × 10^4
		% reductions§	
None	NMS + C	68 ± 10	40 ± 17
α-Qa-1 + C	NMS + C	45 ± 6	21 ± 4
None	α-Qa-1 + C	61 ± 11	34 ± 7
None	α-Thy-1 + C	0	0

* Cells of the indicated T-cell population + 5 × 10^6 B cells (selected with α-Thy-1.2 + C × 2) were incubated with 5 μg/ml Con A + 3 × 10^6 SRBC, and assayed for PFC on day 5.

‡ Graded numbers of the indicated T-cell population + 10^7 unprimed spleen cells + 3 × 10^6 SRBC were cultured for 4 days.

§ 100 − mean PFC count per culture/mean PFC count per control culture.

after stimulation by Con A and were then added in graded numbers to 10^7 spleen cells. Table IV (B) shows that although Qa-1$^+$ cells contribute to the initiation of Ts activity by Con A (line 1 compared to line 2), Ts activity, once generated, was no longer susceptible to elimination by α-Qa-1 + C (line 1 compared to line 3). These data, taken together, indicate that Qa-1$^+$ cells are required for generation of antigen-induced and, to a lesser extent, for optimal Con A-induced Ts activity: once generated, Con A-induced suppressive activity does not require the continuous presence of Qa-1$^+$ cells while SRBC-induced suppressive activity does.

Representation of Qa-1 Phenotypes Within Various T-Cell Sets (Table V). Approximately 70% of Thy-1$^+$ cells are Qa-1$^+$. Since this Thy-1$^+$:Qa-1$^+$ set is larger than any of the Lyt sets estimated for B6 spleen and lymph node cells (33% Lyt-1; 5–10% Lyt-23; 50% Lyt-123; expressed as proportions of the Thy-1$^+$ population [9]), Qa-1 must be expressed on more than one Lyt-defined T cell set. To determine which sets contain Qa-1$^+$ cells, we enumerated B6-*Tla^a* peripheral LNC by cytotoxicity assays with α-Qa-1, α-Lyt-1.2, and α-Lyt-2.2 sera either alone or in combination.

The combined antiserum method indicates a small proportion of Lyt$^-$:Qa-1$^+$ cells, because more cells were lysed by the α-Lyt plus α-Qa-1 combinations than by any α-Lyt serum alone. It is acknowledged that lysis with two antisera may be more efficient than lysis with either antiserum alone, and that this would stimulate a proportion of cells expressing only one of the two antigens. But this is unlikely to be the main explanation of the apparent Lyt$^-$:Qa-1$^+$ cell set, because a small proportion of Thy-1$^-$:Qa-1$^+$ cells is detectable in normal (2) and in LPS-stimulated populations. That issue aside, the data shown in Table V are our estimations of the phenotypes

TABLE V

Representation of Qa-1$^+$ Phenotypes among Lyt Cell Sets

Designation	Thy-1$^+$ cells treated with	Lysis (cytotoxicity index)*
		%
A	α-Qa-1 + C	78
B	α-Lyt-1 + C	90
C	α-Qa-1 + α-Lyt-1 + C	100
D	α-Lyt-2 + C	69
E	α-Lyt-2 + α-Qa-1 + C	89
F	α-Lyt-1 + α-Lyt-2 + C	98
	Lyt Sets implied by these data	
G	α-Lyt-1 = 29	(F-D)
H	Lyt-23 = 8	(F-B)
I	Lyt-123 = 61	(B-G or D-H)

Qa-1 Distribution within the Lyt sets implied by these data:

Observation	Inference
Cells bearing Qa-1 = 78% (A)	
Qa-1 is expressed on more T-cells than belong to any single Lyt set	Qa-1 is expressed on more than one Lyt set
Cells bearing both Qa-1 and Lyt-1 = 68% (A + B − C)	
Qa-1 is expressed on more Lyt-1$^+$ cells than belong to either the Qa-1 or the Lyt-123 sets	Qa-1 is expressed on some members of both Lyt-1 and Lyt-123 sets
Cells bearing both Qa-1 and Lyt-2 = 58% (A + D − E)	
Qa-1 is expressed on 84% of Lyt-2$^+$ cells	At least 82% of Lyt-123 cells are Qa-1$^+$

* All cytotoxic indices and thus all other percentages are expressed as percent of Thy-1$^+$ cells.

and proportions of cell sets identified by the Thy-1, Lyt, and Qa-1 systems. Accordingly we believe the Lyt-123 cell set to be approximately 80% Qa-1$^+$, and that the Lyt-1 set comprises Qa-1$^+$ and Qa-1$^-$ subsets in about equal proportions. The precise proportion of Qa-1$^+$ cells within the Lyt-23 could not be reliably evaluated because of the relatively small proportion of the Lyt-23 set in the T cell population.

Discussion

The genetic disparity involved in the immunization (B6 × A-Tla^b) anti-A (Tla^a), which defines the Qa-1 system, concerns a region of chromosome 17 reaching from a crossover point between H-$2D$ and Tla and extending to a presently unmarked point beyond Tla. It is already clear that genes in this region define several cell surface components, including the TL antigens of thymocytes, histocompatibility antigens responsible for skin graft incompatibility, and the group of serologically defined antigens collectively termed Qa. Qa itself has already been shown to encompass at least three systems, Qa-1, Qa-2, and Qa-3 (10, 11), by serological analysis of Flaherty's B6.K1 and B6.K2 strains derived from different recombinants between H-$2D$ and Tla. Qa-2 and Qa-3 do not enter the picture as far as the present study is concerned because B6 and A (the allele donor of the recombinant congenic B6-Tla^a strain) have so-far indistinguishable Qa-2 and Qa-3 types.

It is impossible at the moment to say how many cell surface components Qa-1 may comprise, nor whether each belongs to a different program that subclassifies the Lyt and perhaps other lymphocyte sets. Although the immunization of (B6 × A-Tla^b) mice is performed with the TL$^+$ leukemia ASL1, it does not follow that ASL1

expresses all Qa-1 antigens that may be represented in the antiserum, because splenic ASL1 cell suspensions include a variety of normal A strain cells. Although analysis is as yet far from complete, the Qa-1 system is already of considerable value because it distinguishes a population of lymphocytes that is not defined by any other antigenic system, according to three criteria: (a) representation of Qa-1 cells among T cell sets defined by Lyt phenotypes, (b) the profile of responses to mitogens exhibited by lymphocyte populations depleted of Qa-1$^+$ cells, and (c) the profile of immune responses of lymphocyte populations depleted of Qa-1$^+$ cells.

Mitogens, as polyclonal activators of lymphocytes, have been a valuable aid in the study of lymphocyte function and the dissection of lymphocyte sets. In this respect it is noteworthy that Qa-1$^+$ cells, which compose part of the Lyt-1 and Lyt-123 sets (their representation in the Lyt-23 set is unknown), are essential for significant responses to PHA and are required for optimal responses to Con A. By the criterion of SRBC PFC production by spleen cells, Con A induces both helper and suppressor functions (6, 12) and has been shown to stimulate all three Lyt T cell sets (6); whereas PHA appears to induce mainly suppression but not help, and other studies have indicated that optimal response to PHA, unlike Con A, may require Lyt-123 cells (13). It may be that Con A can induce Lyt-123$^+$:Qa-1$^+$ cells to become Lyt-23$^+$:Qa-1$^-$ suppressor cells; in the absence of Lyt-123 cells Con A can activate populations enriched for Lyt-23 cells to express suppressive activity. Thus Con A-activated suppression of the α-SRBC PFC response was reduced by elimination of Qa-1$^+$ cells during the generative phase, at a time when amplifiers or precursors (or both) would most likely be needed. This was particularly evident when Con A-induced inhibition depended upon rapid generation of suppressor cells during a 5 day in vitro primary response (Table IV, A). Once generated, Con A-induced suppressor cells were insensitive to elimination of Qa-1$^+$ cells. By contrast Qa-1$^+$ cells were evidently required for both generation and effector function of SRBC-induced suppression of the α-SRBC response. This may imply that SRBC-specific Qa-1$^+$ cells generate additional suppression during restimulation by SRBC in the assay cultures.

In any event, the participation of Qa-1$^+$ cells in the regulation of the SRBC antibody response is a remarkable feature of the Qa-1 system and is primarily reflected in the generation of a T-cell suppressor (Ts) population (14–16). This is the evident reason why elimination of Qa-1$^+$ cells so greatly augments the primary response to SRBC in vitro. Recent experiments have shown that cells of Lyt-123:Qa-1$^+$ phenotype can be induced by antigen-stimulated Lyt-1:Qa-1$^+$ cells to develop substantial feedback suppressive activity (15, 16). We no not know (a) whether Lyt-123:Qa-1$^+$ cells are accessories needed to assist in optimal generation of suppressor-effectors, or give rise directly to suppressor-effectors after induction by Lyt-1:Qa-1$^+$ cells; nor (b) whether Lyt-1 inducer cells and resting Lyt-123 cells express identical Qa-1 region gene products.

The prominence of Qa-1 cells in suppression of the SRBC antibody response in PFC assays contrasts with the minimal influence of Qa-1$^+$ cells in MLC assays, and in both the generation and effector function of cytotoxic lymphocytes active in CMC assays. Evidently alloreactive prekiller and killer cells are Lyt-23$^+$:Qa-1$^-$. Whether the same applies to the generation and effector function of cytotoxic lymphocytes generated against virally or chemically modified cells remains to be seen; this is of special interest because in this case Lyt-123 precursors are necessary for generation of optimal numbers of Lyt-23 cytotoxic effector cells (17).

Summary

The antiserum (B6 × A-Tla^b) anti-A (Tla^a) defines several TL antigens expressed exclusively on thymocytes. When reacted with peripheral lymphocytes, the same antiserum defines another antigenic system, provisionally termed Qa-1. The genotypic disparity distinguishing the recipients and donors in this immunization comprises a section of chromosome 17 extending from a crossover point between H-2D and Tla to a presently unmarked point beyond Tla. Therefore although Qa-1 may constitute a single cell surface component, it is equally probable that the Qa-1 system defines two or more cell surface components determined by genes in this region, each of which may be expressed on a different cell set.

Cytotoxicity assays indicate that Qa-1 antigen is expressed on Lyt-1 cells and Lyt-123 cells, and may serve to subclassify these two cell sets; it is not known whether Qa-1$^+$ cells may occur within the small Lyt-23 set. There may also be a cell set with the phenotype Thy-1$^-$:Qa-1$^+$.

Another distinctive feature of the Qa-1 system is the characteristic profile of responses to mitogens exhibited by spleen cell populations from which Qa-1$^+$ cells have been eliminated; in conventional assay of [^{3}H]thymidine incorporation the response to lipopolysaccharide was essentially unchanged, the response to phytohemagglutinin M (PHA-M) was virtually abolished, and the response to concanavalin A (Con A) was reduced by 40%.

The third distinctive feature of the Qa-1 system is the characteristic profile of changes which elimination of Qa-1$^+$ cells produces in tests of immune function in vitro: (a) proliferation, measured by [^{3}H]thymidine incorporation, in mixed lymphocyte culture (MLC) with major histocompatibility complex (MHC)-incompatible stimulator cells, was not affected. (b) in tests of cell-mediated cytotoxicity (CMC) of MHC-incompatible target cells, neither the generation nor the effector functions of cytotoxic lymphocytes was affected, implying that Lyt-23 prekiller and killer cells are Qa-1$^-$. (c) primary and secondary responses to SRBC were considerably augmented, suggesting that Qa-1$^+$ cells may be responsible for suppression in this test system. (d) accordingly the suppression of the anti-sheep erythrocyte (SRBC) response normally engendered in spleen cells by culture with SRBC was profoundly reduced by elimination of Qa-1$^+$ cells, either before or after culture. (e) the suppression of the anti-SRBC response normally engendered in spleen cells cultured with Con A was reduced by removal of Qa-1$^+$ cells before but not after culture with Con A.

Although analysis is as yet far from complete, the Qa-1 system should already be of considerable value because it distinguishes a population of lymphocytes that is not defined by any other antigenic system, according to three criteria: (a) representation of Qa-1 cells among T-cell sets defined by Lyt phenotypes, (b) the profile of responses to mitogens exhibited by lymphocyte populations depleted of Qa-1$^+$ cells, and (c) the profile of immune responses of lymphocyte populations depleted of Qa-1$^+$ cells.

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