ALTERATION OF CLONAL PROFILE

I. Effect of Sublethal Irradiation on the Responses to Phosphorylcholine in BALB/c Mice

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Antibody responses specific for defined epitopes consist of a heterogeneous array of monoclonal, idiotypically specific antibodies. Restricted heterogeneity of antibody responses has been seen for a number of epitopes: streptococcal vaccines (1, 2), dextran (3), and several different haptens (4–6). Dominance by a single idiotype has been observed for a dinitrophenyl (DNP)[1]-specific clone in adoptive transfer (7) and for the anti-phosphorylcholine response in the intact animal (6). In the humoral response of BALB/c mice to phosphorylcholine (PC), >90% of the plaque-forming cells (PFCs) produce antibodies with the same idiotype as HOPC-8 (H8), a PC-binding BALB/c myeloma protein (6, 8–10). This dominance is found in vivo and in vitro in responses to both thymus-dependent and thymus-independent PC antigens (8, 11). Furthermore, the H8 dominance of the anti-PC response can also be seen in the sera of immunized mice (12). However, BALB/c mice also have the potential to produce anti-PC antibodies with different idiotypes; several idiotypically distinct PC-binding myeloma proteins have originated in this strain (13), and it has been shown that under certain circumstances BALB/c mice can respond to a PC antigen with cells producing H8-negative antibodies (14, 15). Recent studies have uncovered important regulatory influences in the loss and restoration of H8 clonal dominance after transfer of neonatal liver cells to lethally irradiated mice (15). Regulation occurred at the level of the conversion of an idiotypically committed progenitor cell into an antigen-responsive precursor and determined the eventual clonal profile of the response to PC. Similarly, the delay in the emergence of non-H8 clones after neonatal suppression of the H8 clone with anti-idiotypic antibody (14) implicates regulation in the emergence of nondominant clones.

We have previously reported the differential effects of sublethal irradiation on immune responses of adult and neonatal mice (16). Whereas the anti-PC and anti-trinitrophenyl (TNP) responses of adult mice are markedly depressed by prior exposure to radiation, neonates possess a radioresistant population that can quickly

* Supported by the Francis L. Lederer Fellowship.

‡ Supported by a Basil O'Connor Research Starter grant from the National Foundation March of Dimes and by National Institutes of Health grant 1-R01 A1-14530-01; recipient of Research Career Development Award 1K04-A1-00268-01.

[1] *Abbreviations used in this paper:* CRI, cross-reacting idiotype; DNP, dinitrophenyl; anti-H8 id, antisera directed against the idiotype of the HOPC-8 plasmacytoma; PC, phosphorylcholine; PFC, plaque-forming cell; R36a, *Streptococcus pneumoniae* strain R36a; TNP, trinitrophenyl.

restore these immune responses. Thus, ionizing radiation has been used to define a neonatal population of B lymphocytes that are capable of mitigating radiation damage. Although the immediate effects of sublethal irradiation on plaque-forming cells responses have been thoroughly investigated (17) no studies have determined the long-term consequences of ionizing radiation on clonal dominance. In one extended study, Doria et al. (18) demonstrated a profound effect of sublethal irradiation on the kinetics of production and the affinity of anti-DNP antibodies, presumably by removing a radiosensitive regulatory element.

In this paper we have followed the anti-PC response to sublethally irradiated BALB/c mice and analyzed the clonotype of this response by inhibition of plaque formation using antisera specific for the H8 idiotype. Recovery from radiation damage was characterized by an early phase of H8 clonal dominance followed by the emergence of non-H8 clones. Exposure to low doses of irradiation which had minimal effects on anti-PC responses eventually led to the loss of clonal dominance. Our results indicate that there is regulation of the diversity of clonal expression and that the active regulatory element is radiosensitive.

Materials and Methods

Animals. Adult BALB/c mice (8- to 12-wk of age) were obtained from Cumberland View Farms, Clinton, Tenn. Neonatal BALB/c mice were produced in our breeding colony. Adult A/HeJ and CBA/CaJ mice (8- to 12-wk of age) were purchased from The Jackson Laboratory, Bar Harbor, Maine. (CBA/CaJ × BALB/c) F_1 (JBF_1) and (CBA/N × BALB/c) F_1 (NBF_1) hybrid female mice were produced in our breeding colony.

Antigens. Streptococcus pneumoniae strain R36a (R36a) was obtained from the American Type Culture Collection, Rockville, Md. A formalin-killed vaccine which contains the PC antigen was prepared. For immunization, 5×10^8 R36a cocci suspended in 0.2 ml saline were injected intravenously. TNP_{36}-Ficoll was prepared as described by Inman (19). Adult mice were given 10 μg intravenously.

Anti-Idiotypic Antisera. Antisera to the H8 idiotype (anti-H8 id) was produced in A/HeJ mice by multiple injections of the myeloma protein (15). This antisera was absorbed on a column containing normal mouse sera conjugated to Sepharose 4B; the normal sera had been previously absorbed with PC coupled to Sepharose 4B to remove all anti-PC antibodies.

Irradiation. Adult mice were given various doses of total body irradiation from a ^{137}Cs source. Neonatal mice irradiated by the same source were maintained in sterile cages, and the nursing mothers were given water containing Terramycin.

Hemolytic Plaque Assay. A modification of the hemolytic plaque technique was used (20, 21). R36a extract (11, 22) and TNP (23) coupled to sheep erythrocytes were used as target cells.

Results

Specificity of Inhibition of Plaque Formation by Anti-Idiotypic Antisera. Anti-idiotypic antisera raised in A type mice against the BALB/c HOPC8-TEPC15 myeloma proteins have been shown to inhibit anti-PC responses specifically in BALB/c mice (6). Table I presents results of PFC inhibitions with an A/He anti-H8 antiserum which demonstrate that the anti-idiotypic serum used in the present series of experiments can distinguish between H8 (BALB/c) and non-H8 (CBA/CaJ) anti-PC PFC. The anti-H8 id also distinguishes between H8 and non-H8 clones in the same mouse, as shown by the inhibition of PFC produced in F_1 mice. It is important to emphasize that every normal BALB/c mouse that has been tested (2 wk–5 mo of age) exhibits H8 clonal dominance.

Effects of Sublethal Irradiation on the Development of the Dominant H8 Idiotype. Fig. 1 a

TABLE I

Inhibition of Anti-PC PFC by Anti-H8 id Antiserum

Mice	PFC inhibited
	%
BALB/c	99, 98, 99, 97, 98
NBF_1	51, 18, 26, 77, 63, 54, 60, 47, 80, 49
JBF_1	46, 49
CBA/CaJ	12, 0, 0, 0

Anti-PFCs were determined 4 or 5 days after challenge with R36a. Clonotypic analysis was carried out by incorporating a 1:500 dilution of anti-H8 id into the plaquing mixture.

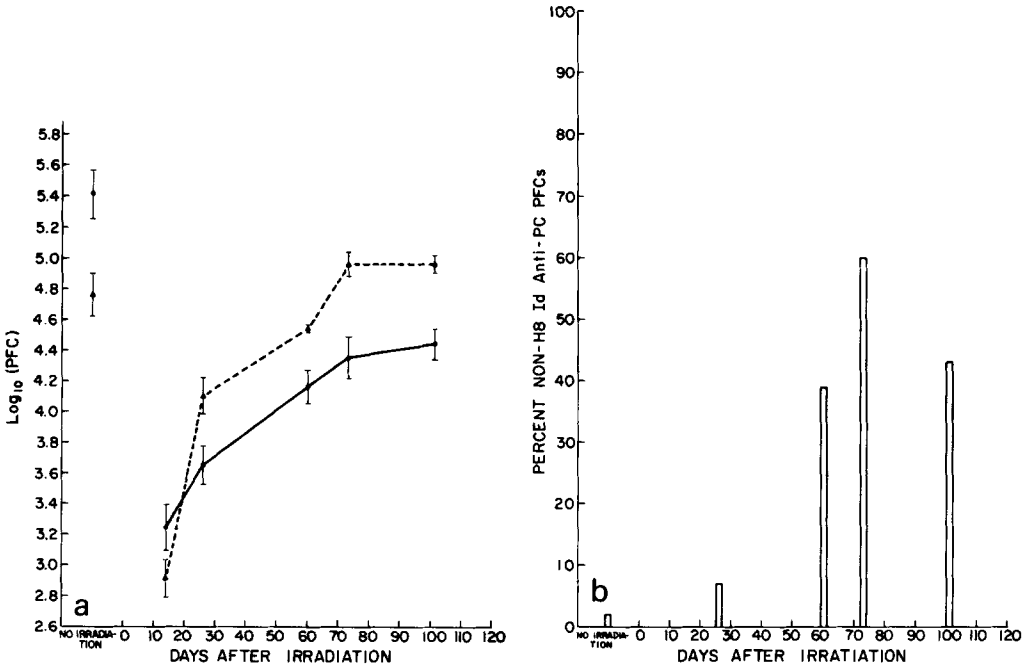

FIG. 1. Recovery of the response to R36a and TNP-Ficoll after sublethal irradiation. (a) Adult BALB/c mice were irradiated with 500 rads, and the direct PFC responses to R36a (●) and TNP-Ficoll (▲) were measured at various times after irradiation. Vertical bars represent the standard error of the geometric mean. (b) Clonotypic analysis was carried out by incorporating a 1:500 dilution of anti-H8 id into the plaquing mixture. Anti-PC PFCs not inhibited by anti-H8 id were 100% inhibited by 10 μM PC. An unirradiated BALB/c mouse was assayed on each day to insure the integrity of the plaque inhibition.

shows the time course of recovery of the response to two antigens by adult BALB/c mice exposed to 500 rads total body irradiation. These mice were immunized with R36a and TNP-Ficoll at various times after sublethal irradiation, and the direct PFC response was measured 5 days later. The clonotypes of the anti-PC responses were analyzed by inhibition of plaque formation using anti-H8 id (24) (Fig. 1 b). Unirradiated animals responded to both antigens, and the anti-PC response showed the characteristic near-total H8 dominance. 14 days after sublethal irradiation the responses to TNP-Ficoll and R36a were markedly reduced; by 26 days both responses

had significantly increased, and the response to PC was dominated by the H8 idiotype. A new pattern of responsiveness was seen at 60 days after irradiation and was stable up to 102 days. Anti-TNP responses had recovered to preirradiation levels but the responses to R36a reached a plateau at a level below that of controls. The reasons for the incomplete recovery of anti-PC responses after irradiation are not apparent and have not been investigated further; it is worth noting, however, that the reconstitution of lethally irradiated BALB/c mice with 10^7 adult spleen cells is more complete for responses to TNP-Ficoll than to R36a (D. R. Kaplan and J. Quintáns, unpublished observations). The more significant aspect of the new pattern of anti-PC responses seen 60–100 days after irradiation is revealed by clonotypic analysis. Whereas the unirradiated BALB/c controls display >90% H8 clonal dominance, a considerable portion of the response of irradiated mice could no longer be inhibited by anti-H8 id. Thus, 500 rads sublethal irradiation resulted in an altered clonal profile for the anti-PC response.

Effects of the Dose of Irradiation on the Immune Response and Clonal Profile. BALB/c mice were exposed to various doses of irradiation, and the direct PFC responses to TNP-Ficoll and R36a were determined 1 or 9 wk after irradiation (Fig. 2). The response at 1 wk was minimally affected by 100 rads, while 300, 400, and 500 rads markedly decreased both responses. By 9 wk the anti-TNP response had recovered from all doses of irradiation; however, the anti-PC response showed a dose-dependent depression of PFCs. Clonotypic analysis of the responses to R36a 10 wk after various doses of irradiation is shown in Table II. Age-matched, unirradiated control mice were H8-dominant. At every dose of irradiation there was at least one mouse that produced H8-negative anti-PC PFCs. Three BALB/c mice out of five given 100 rads total body irradiation 10 wk before the assay produced significant numbers of non-H8 anti-PC PFCs. Even a low dose of irradiation that does not obliterate the immediate H8-dominant anti-PC response can initiate the eventual loss of clonal dominance.

Effects of Neonatal Sublethal Irradiation on the Acquisition of Clonal Dominance. Because

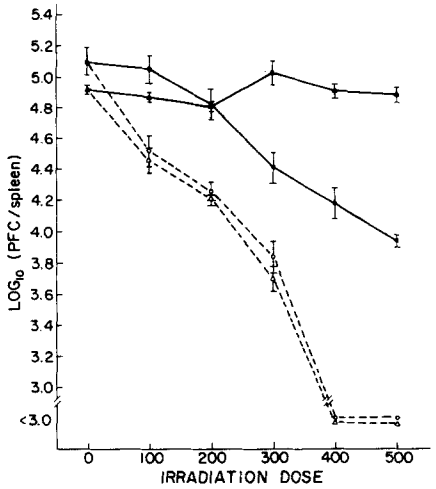

FIG. 2. Recovery of responsiveness to R36a and TNP-Ficoll after various doses of sublethal irradiation. Adult BALB/c mice were given various doses of irradiation. The responses to R36a (circles) and TNP-Ficoll (triangles) were measured 1 (- -) and 9 wk (—) after irradiation. The vertical bars represent standard error of the geometric mean.

TABLE II

Clonotypic Analyses of Individual 10 Wk after Varying Doses of Sublethal Irradiation

Mice	Dose of irradiation	PFC/spleen			Non-H8 id anti-PC PFCs
		Anti-TNP	Anti-PC	Non-H8 id Anti-PC*	
	rads				%
4	—	30,400	82,400	400	<1
		20,800	38,800	400	1
		25,200	81,733	2,000	2
		31,600	55,200	1,200	2
5	100	14,400	47,333	15,200	32‡
		22,600	42,000	4,000	10
		43,000	64,533	100	<1
		11,200	33,733	10,800	32‡
		7,600	10,267	2,400	23‡
5	200	39,800	68,933	400	1
		14,200	40,267	400	1
		30,000	46,533	20,000	43‡
		18,000	30,667	2,000	7
		13,800	34,133	1,200	4
5	300	23,800	27,400	17,200	63‡
		28,000	28,600	6,200	22‡
		45,600	136,933	2,400	2
		18,400	23,333	5,400	23‡
		24,400	36,133	3,800	11
5	400	39,000	5,800	3,750	65‡
		24,800	10,600	2,050	19‡
		28,800	4,167	2,550	61‡
		18,600	4,433	1,400	32‡
		26,100	12,733	2,600	20‡
5	500	65,400	20,000	11,800	59‡
		55,800	20,567	11,450	56‡
		46,400	8,133	3,250	40‡
		119,800	21,733	12,050	55‡
		80,400	25,133	21,250	85‡
		55,400	14,567	10,100	69‡
		125,600	30,433	16,700	55‡

* Clonotypic analysis as in Table I.

‡ Individual mice that produced ≥10% non-H8 clones are marked.

the antigen-responsive precursor B cell for the H8 clone does not appear until 1 wk of age in BALB/c mice (25, and J. Quintáns, unpublished observations), the response to PC provides an excellent opportunity to study the long-term effects of sublethal irradiation on an immature population of B cells. As reported elsewhere, neonates—in contrast to adults—quickly recover the capacity to respond to TNP and PC antigens after 500 rads sublethal irradiation (16). Clonotypic analysis of the anti-PC response demonstrated the initial reestablishment of H8 clonal dominance; however, 4–8 wk after neonatal irradiation a low but detectable level of non-H8 PFCs could be seen in

some of the mice. 4 of 15 mice irradiated at birth had lost H8 clonal dominance; individually their responses were comprised of 19, 31, 57, and 72% non-H8 clones. Four other mice in this same group were marginally nondominant with a 10–15% non-H8 contribution to the anti-PC response. Although the loss of clonal dominance in neonates is not as dramatic as in adult mice, the results clearly indicate that a radiosensitive element contributing to the maintenance of clonal dominance has been damaged in a significant proportion of the neonates. Since irradiation had been given before the acquisition of immunocompetence to PC antigens (25), the irradiation must have acted on a cell other than the antigen-responsive precursor cell.

Discussion

Although the response to PC in BALB/c mice is largely dominated by the H8 id (6, 8–12), clonal dominance can be abrogated in two experimental situations. The neonatal injection of anti-H8 id produces an initial period of unresponsiveness to PC which is followed by the emergence of non-H8 clones (14). The other experimental model involves the syngeneic adoptive transfer of neonatal liver cells; this transfer fails to reconstitute clonal dominance in a lethally irradiated host unless the recipient has been suppressed previously with anti-H8 id (15). Both models involve the manipulation of neonates before the ability to respond to PC is acquired. In this paper we report that sublethal irradiation of adult BALB/c mice leads to the loss of clonal dominance. Whereas >90% of the anti-PC PFCs induced in unirradiated BALB/c mice are H8 positive, responses generated 2 mo after 500 rads total body irradiation contain a considerable number of non-H8 PFCs. A similar pattern is also obtained with radiation doses as low as 100 rads and with the sublethal irradiation of neonates before the acquisition of responsiveness to PC (25, and unpublished observations, D. R. Kaplan and J. Quintáns). The change in the clonal profile that is initiated by sublethal irradiation indicates that a radiosensitive element participates in the regulation of the expression of different anti-PC clones. Even as small a dose as 100 rads can affect the function of this regulatory element so that non-H8 clones can appear. The identity of this element and its mechanism of action are unknown at present. Although we assume the regulatory element is a lymphocyte, the equivalent sensitivities of B and T cells to irradiation make it impossible to use this criterion to further characterize the cell type involved in clonal dominance except to exclude primed T helper cells (17).

T suppressor cells have been implicated in the regulation of B-cell function (26); however, indirect evidence suggests that T cells are not active in the H8 dominance of the anti-PC response. Not only do nude mice with a BALB/c background exhibit clonal dominance, but also nude mice neonatally suppressed with anti-H8 id break suppression with non-H8 clones as quickly as their suppressed Nu/+ littermates (J. Quintáns, unpublished results). Furthermore, in a preliminary experiment the transfer of nylon wool-purified spleen cells into sublethally irradiated (500 rads) BALB/c mice did not prevent the emergence of non-H8 clones. On the other hand our preliminary results show that spleen cells treated with anti-Thy1.2 sera and complement can transfer clonal dominance and the capacity to maintain it; it appears that the regulatory element in H8 clonal dominance is a B lymphocyte.

Although B cells seem to be important in the regulation of the clonal profile of the response to PC, it is most likely that the regulatory cell is not the H8 idiotypic precursor cell. The response induced early after sublethal irradiation displays H8

clonal dominance, but the emergence of these H8 clones does not prevent the appearance of non-H8 clones. Moreover, mice exposed to lower doses of irradiation do not lose their H8 idiotype response even though a loss of dominance eventually ensues. Also, irradiation of neonates before the acquisition of the precursor cell is sufficient to allow the expression of non-H8 clones. Thus, the presence of H8 idiotypic precursor cells does not directly prevent the expression of non-H8 clones. It is possible to suggest, however, that the role of the radiosensitive B cell in the establishment of clonal dominance is to prevent the emergence of non-H8 clones; this cell could contribute to H8 clonal dominance via specific network interactions (27, 28). There is evidence that a similar arrangement of clones exists when H8 expression has been prevented. Neonatally suppressed mice which do not express the H8 clone but produce non-H8 idiotypes (14) have been shown to possess suppressor cells for the H8 idiotype (29). A recent report indicates that these cells are present in nude mice and therefore are not conventional T cells (30). Within the anti-PC response in BALB/c mice, it appears that the expression of one idiotvpe can be associated with the concomitant expression of radiosensitive B cells which prevent alternative idiotypes from being produced. This model is not conceptually different from the allotype-specific interactions described by Bosma and Bosma (31). They showed that normal BALB/c mice had allotype-specific immunity against the CRPC 101 tumor which originated in the CB.20 Ig congenic strain. However, allotype-specific suppressor T cells were involved in this system.

There is evidence in another system for the role of B cells in regulating the idiotype expression of other B cells (32). Eig et al. showed that the transfer of BALB/c lymphocytes producing anti-arsonate antibodies lacking the cross-reacting idiotype (CRI) would completely inhibit the production of the CRI in CAL.20 congenic mice. The evidence presented clearly indicated the B-cell nature of their regulatory element, presumably secondary B cells. Alternatively, another B cell generated concomitantly with the memory B cell might be responsible for suppression of the CRI.

Summary

BALB/c mice exhibit >90% H8 clonal dominance in the immune response to phosphorylcholine. Adult mice exposed to 500 rads were initially unable to produce a humoral immune response to both phosphorylcholine and trinitrophenol antigens, and the direct plaque-forming cell response was slowly regained over several weeks. Clonotypic analysis with antisera directed against the H8 idiotype showed that the H8 clone initially dominated the recovery of the response to phosphorylcholine but that 60 days after the irradiation significant numbers of non-H8 clones could be detected. This same pattern could be seen in mice irradiated with 100 rads, a dose that does not completely abrogate the H8 response to phosphorylcholine. Sublethal irradiation of neonates before they had acquired responsiveness to phosphorylcholine could also eventually lead to the emergence of non-H8 idiotypes. Thus, a radiosensitive element regulates the expression of clonal dominance in anti-phosphorylcholine responses of BALB/c mice.

We are grateful to Paul Griffith and Lucy Gemlo for their expert technical help.

Received for publication 12 June 1978.

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