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SPECIALIZED ANTIGEN-PRESENTING CELLS

Splenic Dendritic Cells and Peritoneal-Exudate Cells

Induced by Mycobacteria Activate Effector

T Cells That Are Resistant to Suppression*

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Topical application of picryl chloride (PCL),¹ the chemically reactive form of the trinitrophenyl (TNP) hapten, regularly induces a contact hypersensitivity reaction (CS) that is of the long-lived type (>3 wk) (1). However, when TNP is coupled to cells in vitro and these cells are used for immunization, three different immunological outcomes may ensue depending on the route of administration and the type of cell to which TNP is coupled. One outcome is the development of specific immunological unresponsiveness (tolerance). This results when TNP-coupled lymphocytes or macrophages (peritoneal-exudate cells, PEC) are injected intravenously and is probably a result of the induction of specific suppressor T cells (1-3). A second outcome is the development of an evanescent form of CS that results when TNP-PEC are administered under conditions that avoid rapid activation of the suppressor circuit. This can be done by pretreating recipient mice with a low dose of cyclophosphamide or injecting the TNP-labeled cells subcutaneously so that the initial contact of antigen with the immune system takes place in the draining lymph nodes rather than in the spleen (1-3).

A third outcome is the development of a long-lived form of CS, similar to that achieved by topical application of PCL. This form of immunity also occurs when TNP-coupled epidermal Langerhans cells are used for immunization, even when these cells are injected intravenously and no cyclophosphamide is used (1). In fact, TNP-coupled Langerhans cells induce CS when coadministered with an immunosuppressive inoculum of TNP-PEC. This implies that antigen presentation on Langerhans cells,

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¹ *Abbreviations used in this paper:* APC, antigen-presenting cell; CFA, complete Freund's adjuvant; CS, contact hypersensitivity reactions; DC, dendritic cells; HBSS, Hanks' balanced salt solution; IFA, incomplete Freund's adjuvant; Mφ, macrophage; PBS, phosphate-buffered saline; PCL, picryl chloride; PEC, peritoneal-exudate cells; TNBS, trinitrobenzene sulfonic acid; TNP, trinitrophenyl.

both in their normal anatomic position (i.e., the skin) or when placed in a different anatomic site (i.e., the spleen), activate effector cells that are relatively resistant to the T cell-mediated suppression induced by the TNP-PEC.

To better understand these findings we asked four relevant experimental questions: (a) Are there other normally occurring antigen-presenting cells with characteristics similar to Langerhans cells? (b) If so, can more stringent criteria be used to establish that the sensitized cells induced by such antigen presentation are indeed resistant to suppression? (c) Can active antigen-presenting cells (APC) be induced into an inflammatory exudate by environmental agents? (d) If so, could the induction of these cells explain the mode of action of certain adjuvants? To approach question a we used TNP-coupled splenic dendritic cells (DC) as a source of immunogen because they represent an "endogenous" component of lymphoid tissue that always express large amounts of Ia and H-2D in the resting state (4), are extremely active as accessory cells (5), and present their own alloantigens in an especially immunogenic way (4). These are attributes that one would expect to find in specialized APC. Question b was approached in two ways: first by coimmunizing mice with a tolerogenic dose of trinitrobenzene sulfonic acid (TNBS), known to induce TNP-specific suppressor cells (6), and second by adoptive transfer of cells immunized by intravenous injection of hapten-labeled APC into naive recipients whose inducible suppressor cell population remains intact. Questions c and d were approached by comparing the immune response produced by TNP-coupled PEC that had been induced by the potent adjuvant complete Freund's adjuvant (CFA), with the response induced by similarly labeled PEC that had been induced with a less potent adjuvant or an adjuvant that leads to an "evanescent" or easily suppressible immune responses such as incomplete Freund's adjuvant (IFA).

We found that the intravenous injection of TNP-DC and TNP-CFA-PEC, in contradistinction to other TNP-coupled cells, produced an immune response that could not be suppressed by coadministration of an intravenous injection of TNBS. Thus, naturally occurring specialized APC, as well as those induced by mycobacteria, activate effector cells that are relatively resistant to suppression.

Materials and Methods

Animals. Inbred CBA/J mice of both sexes were obtained from The Jackson Laboratory (Bar Harbor, ME) at 6–8 wk of age and were rested 1 wk in an air-filtered enclosure before use. In any one experiment, mice were matched for both age and sex.

Reagents. PCL (Eastman Organic Chemicals, Rochester, NY) was recrystallized three times from methanol before use, and was protected from light during storage. TNBS (Eastman Organic Chemicals) was used without further purification. A 10 mM TNBS stock solution was prepared so as to be isosmotic for cells (and, therefore, not to cause significant cell lysis) in the following way: 290 mg TNBS was dissolved in 70 ml phosphate-buffered saline (PBS). The pH of this solution was adjusted to 7.2 by the addition of 5 ml 0.5 M K_2HPO_4 . To bring the final vol to 100 ml, 15 ml of double-distilled water was added, followed by 10 ml of PBS. The final osmolality was 310 mOsmol/liter. This solution was then filtered through a 0.45- μ m Millipore filter (Millipore Corp., Bedford, MA), and stored in 5-ml aliquots at -70°C . Freshly prepared TNBS was generally toxic for cells (>50% cells killed). After 2 wk in the freezer, toxicity was substantially reduced to 10–20%.

Isolation of Splenic DC and Splenic Macrophages ($M\phi$). DC or $M\phi$ were enriched from spleen adherent cells as described previously (7). Spleen adherent cells were cultured overnight, rosetted with antibody-coated erythrocytes, and separated into Fc^- (DC enriched) and Fc^+ ($M\phi$ enriched) components. Bound erythrocytes were removed by lysis in 0.83% ammonium

chloride. The cells in the DC-enriched fraction are >90% DC as assessed by cytologic criteria and absence of several M ϕ and lymphocyte surface markers (4, 7, 8).

Induction of Peritoneal Exudates. PEC were harvested 5 d after intraperitoneal injection of various quantities of stimulators: (a) 0.5 ml H37LRa CFA or IFA (Difco Laboratories, Detroit, MI). Only the oil phase was injected, without emulsification with saline. (b) *Bordetella pertussis* vaccine (2×10^6 dead organisms) (Department of Public Health, Boston, MA). (c) 3 ml thioglycollate medium (Difco Laboratories). Cells were removed from the peritoneum after lavage with 5 ml Hanks' balanced salt solution (HBSS) containing 5% fetal calf serum (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY).

Hapten Labeling of Cells. Cells were suspended in PBS (pH 7.3) at a concentration of 10^8 /ml before the addition of an equal volume of 10 mM TNBS. Trinitrophenylation was carried out for 10 min at 37°C. Further hapten coupling was inhibited by the addition of HBSS containing 10% fetal calf serum. Cells were washed three times with HBSS and viability, as assessed by trypan blue exclusion, was usually 80–90%. Total cell recoveries after hapten coupling ranged between 70 and 90%.

Sensitization of PCL. Mice were sensitized by topical application of 0.1 ml of a 5% PCL solution in absolute ethanol to the shaved abdomen and four footpads.

Induction of Unresponsiveness to PCL with TNBS. Specific unresponsiveness to PCL sensitization was induced by intravenous injection of 4 mg TNBS in 0.2 ml PBS. When hapten-coupled cells were administered at the same time, they were injected in the tail vein. Several minutes later, TNBS was injected into the retroorbital plexus.

Induction of CS Immunity or Unresponsiveness by Cell-bound Hapten. CBA/J mice were injected intravenously with varying numbers of hapten-conjugated cells in 0.2 ml HBSS. 7, 14, and 21 d later, control ear thickness was measured with an engineer's micrometer (L. S. Starrett Co., Athol, MA) before application of 10- μ l doses of a 1% PCL solution (dissolved in olive oil) to each side of both ears. The increment in ear thickness was then measured at various times after challenge. CS responses induced by hapten-conjugated cells, like CS induced by painting with PCL, were absent at 4 h, were evident by 18 h, persisted at 24 h, and by 48 h were decreased but still significant.

Only CS responses 24 h after ear challenge of immunized groups are presented. In each experiment a separate group of nonimmune controls was challenged simultaneously with 1% PCL in olive oil and their resulting 24-h ear swelling responses, which varied from 2–10% increases, were subtracted from that of each immunized group to obtain the net percent increase, which was rounded off to the nearest integer. The statistical significance of the data was calculated using the two-tailed Student's *t* test with $n - 2$ degrees of freedom, where n = the number of mice per experimental group.

Adoptive Transfer of CS. Spleens and lymph nodes were removed from mice that were skin sensitized with PCL 7 d previously. Only spleens from mice immunized for the same length of time by intravenous injection of hapten-coupled cells were removed. Control ear thickness of the untreated CBA/J recipients was measured before mice were injected intravenously with 5×10^7 immune cells. Within 1 h of cell transfer, ears were painted with 1% PCL. The increment in ear thickness was measured 24 h after challenge.

Estimation of the TNP Content of Coupled cells by ^{125}I -MOPC 315 binding. ^{125}I -labeled MOPC 315 prepared as described by Zeldis et al. (9) was the kind gift of Dr. Robert Rosenstein (Department of Pathology, Yale University School of Medicine, New Haven, CT). 5×10^6 hapten-coupled cells were incubated with an excess of ^{125}I -MOPC 315 (sp act $\cong 1.8 \times 10^6$ Ci/mol) for 30 min at 4°C. Cells were washed extensively and transferred to clean tubes for counting. The number of counts bound permits comparison of the relative TNP density on different cell types, provided they are about the same size and are labeled homogeneously.

Results

Studies on CS Induced by Naturally Occurring Cells Coupled with TNP (Table I). Spleen adherent cells were cultured overnight, dislodged from the surface, and separated into DC-rich (Fc^-) and M ϕ -rich (Fc^+) fractions by rosetting with opsonized erythrocytes. TNP-conjugated DC administered intravenously induced a CS reaction that

TABLE I
Induction of Long-lived CS by Intravenous Administration of
TNP-coupled Splenic DC*

Group	TNP-coupled spleen cells injected intravenously		24-h net percent increase in ear swelling \pm SD \ddagger	
	Type	Number	Day 7	Day 21
A	DC	(3×10^5)	11 ± 3	10 ± 2
B	DC	(7×10^4)	4 ± 1	12 ± 0
C	M ϕ	(3×10^5)	0	0
D	M ϕ	(7×10^4)	0.5 ± 1	ND \S

* Results are from two separate experiments: one in which 4-6 mice per group were tested on day 7, and the other in which four mice per group were tested on day 21. $P < 0.05$ for group A vs. C and D on day 7 and groups A and B vs. C on day 21.

\ddagger 24-h net percent increase = 24-h percent swelling response of immunized group that received TNP-coupled cells, minus 24-h percent swelling response of nonimmunized mice that were challenged simultaneously with 1% PCL.

\S Not done.

TABLE II
Dominance of CS Induced by Hapten-coupled Splenic DC
Over a Tolerogenic Dose of TNBS*

Group	Type of TNP-coupled spleen cells (3×10^5) injected intravenously	Tolerogen	24-h net percent increase in ear swelling \pm SD \ddagger	
			Day 7	Day 21
A	DC	—	19 ± 3	19 ± 1
B	DC	TNBS	24 ± 5	23 ± 3
C	M ϕ	—	21 ± 6	0 ± 4
D	M ϕ	TNBS	0 ± 2	4 ± 4

* Table II shows the results of a single experiment in which each experimental group consisted of four mice. These animals were challenged at day 7 and then rechallenged at day 21. $P < 0.05$ for groups A, B, and C vs. D on day 7 and groups A and B vs. C and D on day 21.

\ddagger 24-h net percent increase = 24-h percent swelling response of immunized group that received TNP-coupled cells, minus 24-h percent swelling response of nonimmunized mice that were challenged simultaneously with 1% PCL.

was detectable at 7 and 21 d after immunization. In contrast (in these two experiments), similar numbers of TNP-conjugated M ϕ failed to induce immunity to PCL at day 7 or at day 21. Thus, splenic DC, like epidermal Langerhans cells (1), have a special capacity to present antigen after intravenous injection, such that persistent CS ensues. Splenic M ϕ , like PEC induced by injection of oil or thioglycollate (1), are less well-equipped to induce this type of persistent response.

Immunity Induced by TNP-DC: Effect of Coimmunization with a Tolerogenic Dose of TNBS (Table II). Although TNP-coupled splenic M ϕ failed to induce CS after intravenous injection in the experiments presented in Table I, this was not always the case. Occasionally, these cells induce CS. Because previous work suggested that all Ia⁺ glass-adherent cells could present antigen effectively if suppressor mechanisms were bypassed (1), we considered the possibility that when TNP-coupled glass-adherent

TABLE III
*Induction of CS to PCL by TNP-coupled PEC Induced by Various Activators**

Group	PEC stimulator	24-h net percent increase in ear swelling \pm SD \ddagger	
		Day 7	Day 14
A	None \S	-1 ± 2	ND
B	IFA	5 ± 2	2 ± 5
C	<i>B. pertussis</i> vaccine	2 ± 1	ND \parallel
D	Thioglycollate medium	1 ± 5	0 ± 6
E	CFA	15 ± 3	13 ± 2

* Mice were injected intravenously with 5×10^6 TNP-PEC and 7 or 14 d later their ears were challenged with 1% PCL. The data were compiled from four independent experiments in which each experimental group consisted of four to five animals. Each regimen was tested at least twice. $P < 0.05$ for groups A, B, C, and D vs. E on day 7 and groups B and D vs. E on day 14.

\ddagger 24-h net percent increase = 24-h percent swelling response of immunized group that received TNP-coupled cells, minus 24-h percent swelling response of nonimmunized mice that were challenged simultaneously with 1% PCL.

\S Unstimulated peritoneal cells.

\parallel Not done.

spleen cells induce immunity after intravenous injection, they do so by avoiding suppression. To test this notion, we performed several experiments in which we immunized mice intravenously with TNP-labeled cells and at the same time gave them a tolerogenic dose of TNBS. The results in Table II show that on those occasions when CS is induced by TNP-coupled splenic M ϕ , the response is different from that induced by TNP-DC in two ways: (a) it is completely suppressed by concomitant injection of TNBS and (b) it vanishes by day 21, even when no other tolerogenic stimulus is presented. Most likely, the waning of the response is because of its sensitivity to regulatory suppressive mechanisms. Thus, our results show in two different ways that the immune response induced by TNP-DC is more resistant to suppression than is the response induced by TNP-M ϕ . Furthermore, the failure of TNBS injection to diminish the response in mice immunized with TNP-DC indicates that these cells induce a truly suppressor-resistant response, not that they simply fail to activate suppression.

Studies of CS Induced by TNP-coupled PEC Induced by Mycobacteria (Table III). Intravenous injection of TNP bound to resident peritoneal cells or PEC stimulated by IFA, *B. pertussis* vaccine, or thioglycollate medium failed to induce detectable CS to PCL at day 7 or day 14 after immunization. In addition, subsequent attempts to sensitize these nonresponding mice by skin painting with PCL ~24 h after primary challenge also failed to induce CS (data not shown). Thus, TNP-conjugated PEC that were elicited by these means failed to induce immunity and appeared to induce unresponsiveness when injected intravenously. In contrast, TNP conjugation of PEC stimulated by CFA (containing heat-killed H37Ra mycobacteria) induced CS that was elicited at day 7 and still elicitable at day 14 after intravenous immunization. Thus, TNP-conjugated PEC stimulated by mycobacteria (TNP-CFA-PEC) not only failed to induce unresponsiveness to PCL but had acquired the capacity to induce immunity to PCL when administered by the intravenous route. Furthermore, like CS induced by skin-painting with PCL, or intravenous immunization with TNP-conju-

gated epidermal Langerhans cells (1) or splenic DC (Tables I and II), CS induced by TNP-CFA-PEC was relatively long-lived.

Effect of Varying Cell Numbers on the Induction of CS with TNP-conjugated Cells (Table IV). Intravenous injection of 5×10^7 haptenated spleen cells is a standard protocol for the induction of unresponsiveness to CS (3), whereas only 5×10^6 TNP-CFA-PEC given intravenously was needed to immunize mice for CS. To determine whether this qualitative difference was a function of cell number and/or dose of antigen, varying numbers of TNP-conjugated PEC or spleen cells were injected intravenously into mice that were challenged at day 14. TNP-CFA-PEC were capable of inducing CS at doses of 5×10^7 or 5×10^6 cells but not at a dose of 5×10^5 cells. Hapten-conjugated IFA-PEC or spleen cells, however, failed to induce CS at all doses tested. Thus, the immunizing potential of TNP-CFA-PEC remained intact at higher numbers of cells and disappeared when too few cells were injected. Neither increasing nor decreasing the dose of hapten-conjugated IFA-PEC or spleen cells gave them the capacity to induce immunity by intravenous injection. These results strongly suggest that qualitative and not simple quantitative differences account for the difference in immunizing capacity of the two cell types.

Estimation of Relative TNP Content of Hapten-conjugated Cells (Table V). The data in Table IV are consistent with the idea that induction of CS with TNP-CFA-PEC is not a result of a cellular dose effect. The total amount of TNP injected, however, may be a crucial determinant of immunogenicity. Thus, it was important to quantitate the number of haptenic determinants that were injected into the mice. To address this question, equal numbers of haptenated or nonhaptenated cells were incubated with an excess of ^{125}I -MOPC-315, a TNP-binding myeloma protein, for 30 min at 4°C . After extensive washing, cells were transferred to new tubes for counting. The results (Table V) allow comparison of the average number of counts bound by spleen

TABLE IV
*Induction of CS to PCL by TNP-coupled Cells: Dose Response**

Group	Cells injected intravenously	24-h net percent increase in ear swelling \pm SD on day 14 \ddagger
A	5×10^7 TNP-CFA-PEC	21 ± 5
B	5×10^6 TNP-CFA-PEC	20 ± 3
C	5×10^5 TNP-CFA-PEC	6 ± 3
D	5×10^7 TNP-IFA-PEC	0 ± 0
E	5×10^6 TNP-IFA-PEC	3 ± 4
F	5×10^5 TNP-IFA-PEC	4 ± 4
G	5×10^7 TNP-spleen cells	0 ± 4
H	5×10^6 TNP-spleen cells	3 ± 2
I	5×10^5 TNP-spleen cells	4 ± 3

* Table IV shows the results of a single experiment in which each experimental group consisted of four animals. $P < 0.05$ for groups A and B vs. C, D, E, F, G, H, and I.

\ddagger 24-h net percent increase = 24-h percent swelling response of immunized group that received TNP-coupled cells, minus 24-h percent swelling response of nonimmunized mice that were challenged simultaneously with 1% PCL.

TABLE V
Binding of ^{125}I -MOPC 315 to Haptenated vs. Nonhaptenated Cells*

Group	Cell type	Hapten coating	Counts per minute bound
A	CFA-PEC	TNP	2,780
B	CFA-PEC	None	215
C	IFA-PEC	TNP	2,686
D	IFA-PEC	None	62
E	Thioglycollate-PEC	TNP	12,515
F	Thioglycollate-PEC	None	746
G	Spleen cells	TNP	7,046
H	Spleen cells	None	451
I	None	None	514

* 5×10^6 haptenated or nonhaptenated cells were incubated with an excess of ^{125}I -MOPC-315 (sp act $\cong 1.8 \times 10^6$ Ci/mol) for 30 min on ice. Results are the average of triplicate tubes.

TABLE VI
Failure of Nonhaptenated CFA-PEC to Provide an Adjuvant Effect for TNP Presented on IFA-PEC*

Group	Cells injected intravenously	24-h net percent increase in ear swelling \pm SD at day 21 \ddagger
A	5×10^6 TNP-CFA-PEC	15 ± 4
B	5×10^6 TNP-IFA-PEC	2 ± 3
C	5×10^6 TNP-IFA-PEC + 5×10^6 CFA-PEC	3 ± 3

* Cells were mixed and injected in a vol of 0.4 ml HBSS via the tail vein. Each experimental group consisted of four mice. $P < 0.05$ for group A vs. B and C.

\ddagger 24-h net percent increase = 24 h percent swelling response of immunized group that received TNP-coupled cells, minus 24-h percent swelling response of nonimmunized mice that were challenged simultaneously with 1% PCL.

cells or PEC stimulated by CFA, IFA, or thioglycollate medium. Although spleen cells and thioglycollate-stimulated PEC bound 2–5 times more counts than PEC stimulated by CFA or IFA, it was clear that binding of MOPC-315 to CFA or IFA-PEC was equivalent. Since CFA and IFA-PEC were roughly similar in size, as determined by microscopic inspections, the differences in immunizing potential between these cells cannot be accounted for simply on the basis of antigen (hapten) dose.

Requirement for Haptenation of the CFA-stimulated PEC for Antigen Presentation (Table VI). To determine whether the differences in immunity induced by hapten-conjugated CFA and IFA-PEC were actually related to antigen presentation by these cells or result from an ill-defined adjuvant effect of the mycobacteria on bystander cells, we tested whether hapten conjugation to the CFA-stimulated PEC was obligatory for

TABLE VII
*Dominance of CS Induced by TNP-CFA-PEC:
 Effect of Coimmunization with TNBS*

Group (4 mice/ group)	Immunogen*	Tolerogen‡	24-h net per- cent increase in ear swelling ± SD at day 7§
A	TNP-CFA-PEC	—	15 ± 6
B	TNP-CFA-PEC	TNBS	21 ± 4
C	TNP-IFA-PEC	—	7 ± 1
D	TNP-IFA-PEC	TNBS	2 ± 1
E	5% PCL topically	—	27 ± 3
F	5% PCL topically	TNBS	4 ± 4

* 5×10^6 TNP-coupled cells were injected intravenously via the tail vein.

‡ Several minutes after injection of cells, 4 mg TNBS was injected into the retroorbital venous plexus.

§ Each experimental group consisted of four to five mice. $P < 0.05$ for groups A, B, and C vs. D. 24-h net percent increase = 24-h percent swelling response of immunized group that received TNP-coupled cells or PCL topically, minus 24-h percent swelling response of nonimmunized mice that were challenged simultaneously with 1% PCL.

|| 4 mg TNBS was injected intravenously 7 d before topical application of 5% PCL.

TABLE VIII
*Adoptive Transfer of CS to PCL Induced by TNP-CFA-PEC
 or TNP-IFA-PEC**

Group	Immunization	24-h net percent increase in ear swelling ± SD at day 7‡	
		Donor	Recipient
A	5% PCL	30 ± 6	15 ± 5
B	TNP-CFA-PEC (5×10^6)§	16 ± 3	7 ± 2
C	TNP-IFA-PEC (5×10^6)	10 ± 1	0 ± 3
CS at day 14			
		Donor	Recipient
D	5% PCL	31 ± 5	11 ± 5
E	TNP-CFA-PEC (5×10^6)	23 ± 1	13 ± 6
F	TNP-IFA-PEC (5×10^6)	0 ± 5	0 ± 3

* Table VIII shows data combined from three independent experiments in which donor experimental groups consisted of four animals, and recipient experimental groups consisted of six animals. $P < 0.05$ for groups A, B, C, D, and E vs. F (donors) and $P < 0.05$ for groups A and B vs. C and groups D and E vs. F (recipients).

‡ 24-h net percent increase = 24-h percent swelling response of immunized groups that were actively sensitized or received immune cells, minus 24-h percent swelling response of nonimmunized mice that were challenged simultaneously with 1% PCL.

§ Number of cells injected intravenously is in parentheses.

antigen presentation. The results in Table VI show that the inability to induce persistent immunity with TNP-IFA-PEC cannot be overcome by coadministration of nonhaptened CFA-stimulated PEC.

Effect of Coimmunization with TNP-PEC and a Tolerogenic Dose of TNBS (Table VII). Although TNP-IFA-PEC failed to induce CS after intravenous injection in the experiments presented in Tables III, IV, and VI, on some occasions these cells (like splenic M Φ [Table II]) can induce CS. Thus we performed a series of experiments in which we coadministered a tolerogenic dose of TNBS to test whether CS induced by TNP-IFA-PEC was more sensitive to suppression than that induced by TNP-CFA-PEC. Intravenous injection of 4 mg TNBS 7 d before a sensitizing skin-painting with PCL blocked the induction of CS to PCL (groups E and F). Nonetheless, when TNP-CFA-PEC were injected into mice simultaneously with this dose of TNBS, CS at day 7 was undiminished when compared with mice immunized with TNP-CFA-PEC alone (groups A and B). In this experiment, hapten-conjugated IFA-PEC were very weakly immunogenic, in contrast to earlier experiments in which they failed to induce CS by the intravenous route. However, coadministration of TNBS prevented the development of this CS. Thus, although both IFA- and CFA-stimulated PEC provided effective antigen presentation, CS induced by TNP-CFA-PEC was not only quantitatively greater, but was also insensitive to suppression by coadministration of TNBS.

The Ability to Adoptively Transfer CS Induced by Hapten-conjugated CFA vs. IFA-PEC (Table VIII). Since the initial demonstrations by Dresser (10) and Celada (11) it has been shown repeatedly that some immune responses are hard to adoptively transfer without inactivating the recipient's suppressor T cells (12). Thus, we reasoned that if the effector cells of CS that arise after immunization with TNP-CFA-PEC are truly suppressor resistant, then they should be better able to transfer CS than effector cells that are raised by immunization with TNP-IFA-PEC. The results shown in Table VIII demonstrate that the CS elicited at both day 7 and 14 in mice topically sensitized with PCL could be adoptively transferred into normal recipients. The magnitude of the ear swelling response induced by TNP-CFA-PEC was less than that obtained by skin painting, but the efficiency of transfer was at least as good. In contrast, TNP-IFA-PEC induced a moderate level of CS at day 7 that was no longer detectable by day 14. Furthermore, day-7 CS that was induced by TNP-IFA-PEC was more sensitive to suppressor mechanisms that prevent cell transfer of CS than were T cells induced by TNP-CFA-PEC.

Discussion

The results we have presented in this manuscript taken together with previous results (1) suggest that the type of cell that presents antigen to the immune system is of great importance in determining both the qualitative and quantitative aspects of the immune response that an antigen will stimulate. We have identified two major classes of APC that can be distinguished from one another by the sensitivity to suppression of the immune response they induce.

Previous studies have shown that TNP-labeled Ia⁺ PEC can induce evanescent CS if injected subcutaneously. However, induction of CS by administration of these cells by the intravenous route requires pretreatment of the host with cyclophosphamide (1). On the other hand, TNP-labeled epidermal Langerhans cells injected by either route are immunogenic (1). Thus, the proficiency of Langerhans cells in presenting

antigen intravenously did not depend upon avoidance of suppressor cell induction in the spleen (accomplished by subcutaneous injection) or elimination of suppressor cells by cyclophosphamide (13), but was a result of their induction of suppressor-resistant effector cells (1).

We have now shown that another naturally occurring APC type, i.e., splenic DC, can act like Langerhans cells and that this specialized capacity to present antigen can also be induced in peritoneal exudates by mycobacteria. We have further emphasized the T suppressor resistance of the CS response induced by DC- or CFA-induced PEC by showing that the immune response that occurs after they present TNP to the system is: (a) long lived, (b) not affected by coadministration intravenously of a highly tolerogenic dose of TNBS, and (c) can be adoptively transferred to antigenically naive recipients. This last point is particularly helpful in determining the mechanism by which these specialized APC work because of the recent findings of Iverson et al.², who showed that adoptive transfer of CS to normal recipients required a cell that could protect T effector cells from the action of host suppressor T cells that are activated by the adoptively transferred cells. These protector cells can be distinguished from other T cells by their expression of a unique cell surface antigen coded for by genes in the I-J subregion of the major histocompatibility complex and have been referred to as contrasuppressor cells (14). Adoptive immunity does not require the presence of these contrasuppressor cells when the recipient's suppressor system has been diminished by treatment with low doses of cyclophosphamide.

All three of the distinctive features of the CS response induced by the specialized APC we have tested can be explained by the induction of a response that is relatively resistant to normal suppressor mechanisms. The results of the adoptive transfer experiments indicate that the resistance to suppression is mediated, at least in part, by contrasuppressor cells. Taken together then, our results suggest that specialized APC not only activate helper and/or effector T cells, but that they also activate the cells that comprise the contrasuppressor circuit (14, 15).²

The question of how these specialized APC induce suppressor-resistant effector cells and/or activate contrasuppressor cells remains to be determined. The amount of Ia antigen that APC express on their surface or the rate at which they shed these antigens into the local environment may influence the type of T cell subset with which they interact. Thus, some T cell subsets may have to recognize more self-major histocompatibility complex-encoded antigen on the APC to be activated. Alternatively—or additionally—specialized APC may present, in addition to antigen, other signals that activate special T cell gene programs, including the one that makes T cells resistant to suppression. This could be accomplished by modulating the numbers of receptors that T cells express for suppressor factors. Mechanisms involving Ia antigens are particularly attractive because: (a) intensive immunization with mycobacterial adjuvants induces increased numbers of Ia⁺ macrophages (5), (b) splenic DC express large amounts of Ia (4, 7), and (c) agents that inhibit turnover of Ia on cell surfaces diminish the ability of the treated cells to stimulate immune responses.³ However, Ia positivity per se cannot explain our results. Ia⁺ thioglycollate-induced TNP-PEC are needed to

² Iverson, G. M., W. Ptak, D. R. Green, and R. K. Gershon. The role of contrasuppression in the adoptive transfer of immunity. Manuscript submitted for publication.

³ Emerson, S. G. J. Pretell, W. Stock, G. M. Iverson, D. Murphy, and R. K. Gershon. Treatments which influence the expression and shedding of Ia antigens inhibit the ability of cells to stimulate mixed lymphocyte reactions. Manuscript submitted for publication.

induce even the short-lived form of CS, and treatment with anti-Ia and complement eliminates this. Furthermore, when these same Ia⁺ cells are injected intravenously, they induce tolerance (1). Finally, the day-4 IFA- and CFA-PEC that induce the two types of CS do not grossly differ in numbers of Ia⁺ cells (~5%), as judged by indirect immunofluorescence in two separate experiments (our unpublished observations).

Another possibility that must be considered stems from the finding that splenic DC express high levels of H-2D as well as I-A (4). Because cells in suppressor circuit appear to be preferentially activated by allogeneic H-2D (16), it would not be at all surprising if contrasuppressor cells also had a predilection for reacting with antigen in association with H-2D. Thus, the reason that DC are such exceptionally good APC may be a result of their high levels of Ia, which help them activate helper cells, and also of their high levels of H-2D, which help them present signals to the contrasuppressor system.

In the search for variables that might be important in explaining the differential ability of hapten-labeled CFA vs. IFA-activated PEC to induce persistent CS reactions, the numbers of APC inoculated and quantitation of the number of haptenic groups per APC were examined. These factors, however, were not able to account for the observed differences. CFA may cause an influx of DC into the peritoneal cavity, which has been identified in bacillus Calmette-Guérin-elicited exudates (17), and some of the adjuvant properties of CFA may be attributed to the inducibility of this APC population. However, we should also emphasize that although TNP-labeled DC and TNP-CFA-PEC both have similar effects on the immune system, the mechanism by which they work need not be precisely the same.

In sum, the results we have presented indicate that one of the important variables in determining the type of an immune response that an antigen will induce depends on the type of cell that presents the antigen to the regulatory T cell system. When some specialized APC present the antigen, then the immune response will be, if not quantitatively stronger, certainly longer-lasting and harder to suppress. These attributes could be responsible for important qualitative changes that occur during the course of an immune response such as the production of high affinity antibodies that take a long time to be generated and that, being highly sensitive to T cell-suppressor mechanisms (18, 19), must be protected during the maturation and/or differentiation of precursor cells.

Summary

We have tested the ability of several types of trinitrophenyl (TNP)-labeled Ia⁺ cells to induce contact hypersensitivity (CS) after intravenous injection. Most labeled cell types (spleen cells, splenic macrophages, various types of peritoneal-exudate cells) not only fail to induce CS after this type of inoculation but, rather, activate T suppressor cells leading to specific immunological tolerance. Occasionally, some of these immunizing cells managed to bypass the T suppressor system and induced CS. In those cases the response was short-lived and could be blocked by concomitant injection of trinitrobenzylsulphonic acid (TNBS), a potent inducer of T suppressor cells.

In sharp contrast to these results, TNP-labeled splenic dendritic cells and TNP-labeled peritoneal-exudate cells induced by complete Freund's adjuvant had the following distinctive features: (a) They were always able to sensitize when injected intravenously, and the degree of sensitization they produced was roughly equivalent

to that achieved by cutaneous application of picryl chloride, the chemically reactive form of TNP. (b) The response they elicited was long lived (i.e., lasted for >3 wk). (c) Their sensitizing capacity could not be blocked by the concomitant injection of TNBS. (d) They elicited a response that could be adoptively transferred to untreated, normal recipients.

These results indicate that the type of cell that first presents antigen to the immune system plays an important, even essential, role in determining the strength and duration of the subsequent immune response. In particular, the results suggest that some special antigen-presenting cells can induce a response that is relatively resistant to host suppressor mechanisms. Evidence that they do so by activating contrasuppressor cells is discussed.

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