LACK OF A CORRELATION BETWEEN CELL-MEDIATED IMMUNITY TO THE CARRIER AND THE CARRIER-HAPTEN HELPER EFFECT

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It is generally recognized that the effector cells in delayed-type hypersensitivity reactions are thymus-derived (T) cells (1, 2). There is also ample evidence that the "helper" cells involved in the carrier-hapten cooperative initiation of the antibody response are of thymus origin (3). Recently, there has been some evidence that these two T-cell functions are mediated by the same cell. For example, both cell-mediated immunity and helper-cell function have closely related dose-response curves (4), kinetics of sensitization (4), and susceptibility to antibody suppression (4–6). In addition, helper cells (7) and cellular immune cells (8) appear to have a similar anatomical distribution and comparable levels of radiation resistance (1). In contrast to these findings, in the present communication we present data using a classic hapten-carrier system where there appears to be a lack of a correlation between delayed hypersensitivity to the carrier and the carrier-hapten helper effect.

Materials and Methods

Animals.—Female outbred Wistar rats (strain J) of 9–12 wk of age were used.

Antigens.—Flagellin (FIN) and polymerized flagellin (POL) from *Salmonella adelaide* (Strain SW 1338; H antigen, fg; 0 antigen, 35) were prepared as described previously (9). Hemocyanin (HCY) was isolated from *Jasus lalandii*. Bovine serum albumin (BSA) was obtained from Armour Pharmaceuticals, Eastbourne, England. The acetoacetylated derivative of *S. adelaide* flagellin (AFIN) was prepared as described in an earlier publication (10). The AFIN used in this paper had 17 acetoacetyl groups attached to each FIN molecule and the "relative antigenic activity" (Krel) was 6.8×10^{-3} (10). FIN substituted with 5, 9, 19, 29, and 32 DNP groups/mol was prepared (10). Also, $DNP_{15}HCY$ and $DNP_{20}BSA$ were synthesized.

Immunization Procedures.—Groups of rats (7–8 per group) were injected intradermally in the flanks with 1 μg of POL, FIN, or AFIN, either in saline or emulsified in Freund's complete adjuvant (FCA). Control rats were injected with FCA alone. 28 days later anti-DNP antibody responses were elicited by the injection into the right hind footpads of 1 μg of one of the DNP-FIN derivatives (ranging from unconjugated FIN to DNP_{32}-FIN) or DNP_{15}-HCY in 50 μl saline. Rats were bled 7 days after challenge and the antibody responses to DNP estimated.

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Antibody Estimations.—Antibodies to flagellin were estimated by hemagglutination using sheep erythrocytes sensitized with *S. adelaide* POL by a chromic chloride procedure (12). The same procedure was used for estimating antibody to DNP except that in this case the sheep erythrocytes were sensitized with $DNP_{20}BSA$. All antibody titers expressed as $\log_2$; tube 1, $\frac{1}{10}$ dilution.

RESULTS

Comparison of the Humoral and Cell-mediated Immunity Induced by the Different Flagellar Antigens.—It has been reported previously that *S. adelaide* flagellin can exist in three states which widely differ in their capacity to induce flagellin-specific delayed-type hypersensitivity (11) (Table I). When flagellin is injected in its polymerized form (POL) into rats, little or no delayed hypersensitivity is induced, whereas when injected in its monomeric state (FIN) substantial levels of delayed reactivity are provoked. If the monomeric form of flagellin is heavily substituted with acetoacetyl groups (AFIN), high levels of delayed hypersensitivity are induced. The three forms of flagellin also differ dramatically in their ability to stimulate a primary antibody response (Table I) (11), such differences being inversely related to the levels of delayed hypersensitivity provoked.

TABLE I

*Comparison of the Serum Antibody Levels and Delayed-Type Hypersensitivity Induced by the Different Flagellar Antigens**

Priming antigen (1 μg)	Serum antibody titer (28 days)		Delayed-type hypersensitivity to flagellin	
	Priming in saline	Priming in FCA	Priming in saline	Priming in FCA
NIL	<0.5	<0.5	0.3 ± 0.2‡	0.6 ± 0.2
POL	9.3 ± 0.4	10.5 ± 0.3	0.7 ± 0.3	1.1 ± 0.2
FIN	6.6 ± 0.3	8.8 ± 0.2	2.5 ± 0.2	2.7 ± 0.1
AFIN	<0.5	0.5 ± 0.2	3.7 ± 0.4	5.9 ± 0.3

* Groups of rats were injected intradermally with 1 μg of the various antigens and 28 days later delayed hypersensitivity reactions were elicited in the hind footpads by the injection of 1 μg of flagellin in saline (11).

‡ Delayed-type hypersensitivity measurements represent 24-h footpad swellings (0.1 mm). All values ± standard errors of the means.

Comparison of the Helper Activity Induced by the Different Flagellar Antigens.—A series of experiments was carried out to compare the ability of POL, FIN, and AFIN to induce helper cells for the cooperative initiation of an anti-hapten (DNP) antibody response (see Figs 1 and 2). Control rats which were injected with either saline or FCA and then challenged with the DNP-FIN derivatives produced little or no detectable anti-DNP antibody 7 days after challenge. In contrast, rats which had been primed with the flagellar antigens before DNP-FIN challenge gave substantial levels of anti-DNP antibody. Such priming occurred whether rats were injected with the flagellar antigens in saline

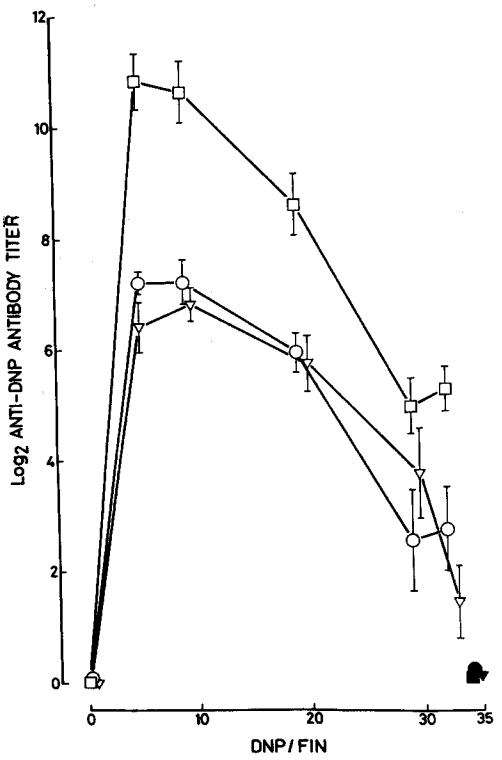

FIG. 1. The anti-DNP antibody response in rats primed with 1 μg of POL (□), FIN (○), or AFIN (▽) in saline and challenged with a range of DNP-FIN derivatives or DNP-HCY (closed symbols) in saline. Antibody titers were estimated 7 days after challenge. Vertical bars represent standard errors of the means. Control rats injected with saline alone failed to produce any detectable anti-DNP antibody 7 days after challenge.

(Fig. 1) or FCA (Fig. 2), and was "carrier specific" as DNP-HCY was unable to initiate an anti-DNP antibody response in these animals. However, the three flagellar antigens differed in the levels of DNP priming which they induced. These differences were especially pronounced in the case of POL-primed rats challenged with lightly substituted DNP-FIN (i.e., DNP_5-FIN, DNP_9-FIN, and DNP_{19}-FIN) where anti-DNP antibody responses were up to 20 times higher than with FIN- or AFIN-primed animals (P values < 0.01) (Figs. 1 and 2). When rats were challenged with heavily conjugated DNP-FIN (i.e., DNP_{29}-FIN and DNP_{32}-FIN) all three flagellar antigens gave similar levels of DNP priming. Rats primed with either FIN or AFIN in saline and challenged with DNP-FIN produced comparable levels of anti-DNP antibody (Fig. 1). However, when these flagellar antigens were injected in FCA, four out of five of the DNP-FIN preparations provoked significantly higher anti-

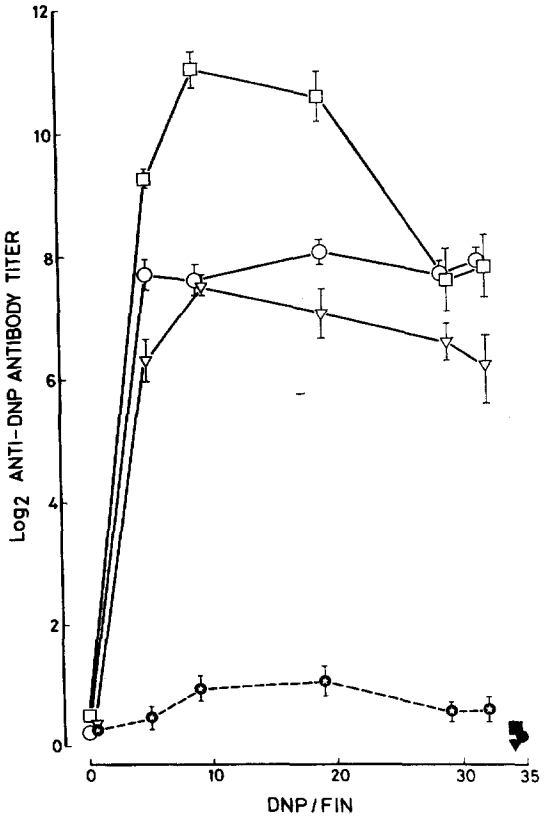

FIG. 2. The anti-DNP antibody response in rats primed with the flagellar antigens in FCA and challenged with a range of DNP-FIN derivatives. For legend see Fig. 1. Control rats were injected with FCA alone and produced small amounts of anti-DNP antibody 7 days after challenge (star symbol).

DNP antibody titers (P values < 0.05) in the FIN-primed rats than in the AFIN-treated animals (Fig. 2).

DISCUSSION

In this paper evidence is presented suggesting that there is no direct correlation between delayed-type hypersensitivity to a carrier and the level of helper cells available for the cooperative initiation of an antihapten antibody response. This evidence was obtained by using three forms of a flagellar antigen, namely POL, FIN, and AFIN, which differed widely in their ability to induce FIN-specific delayed-type hypersensitivity, being in the order AFIN > FIN > POL. In contrast, helper activity seemed to be almost inversely related to delayed hypersensitivity to the carrier, being in the order POL > FIN $\geqslant$ AFIN. This phenomenon has also been observed with the in vitro antibody

response to NIP (4-hydroxy-5-iodo-3-nitro-phenacetyl) sheep erythrocytes by mouse spleen cells (I. Trowbridge, personal communication). It was found that periodate oxidized-acetoacetylated sheep erythrocytes, which induced high levels of delayed hypersensitivity to sheep red cells (13), were much poorer inducers of helper cells for NIP than were the unmodified sheep erythrocytes which induced no detectable delayed responsiveness. Consistent with these findings is the recent report (14) that the number of helper cells available for an antibody response to sheep erythrocytes is substantially reduced when cell-mediated immunity to this antigen is induced.

There are several possible interpretations of the findings presented in this paper: (*a*) Different subpopulations of T cells mediate help and delayed hypersensitivity. Whether these cells are derived from separate precursors or represent different maturation stages of a common precursor is unknown. (*b*) Both helper activity and delayed hypersensitivity is expressed by the same cell but small numbers of these cells induce help whereas large numbers suppress the antibody response. (*c*) Only a small number of antigenic determinants can mediate help whereas many determinants can provoke delayed hypersensitivity. This interpretation would imply that POL is more effective than FIN or AFIN at triggering T cells which recognize "helper determinants". (*d*) The helper effect observed is mediated by B cells and not by T cells. This possibility is unlikely due to the observed thymus-dependence of POL antibody responses in the rat (15).

SUMMARY

The relationship between cell-mediated immunity to the carrier and the carrier-hapten helper effect was studied in the rat by using three forms of the carrier which differed in their capacity to induce carrier-specific delayed-type hypersensitivity. The three carriers were polymerized flagellin (POL), flagellin (FIN), and acetoacetylated flagellin (AFIN), which induced FIN-specific delayed-type hypersensitivity in the order AFIN > FIN > POL. Helper cells for the anti-DNP antibody responses to a range of DNP-FIN conjugates appeared to be almost inversely related to cell-mediated immunity to the carrier, being in the order POL > FIN $\geqslant$ AFIN. These differences occurred whether the carriers were injected in saline or FCA, but were less pronounced with the heavily DNP-conjugated flagellins.

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