IMMUNE RESPONSES AGAINST NATIVE AND CHEMICALLY MODIFIED ALBUMINS IN MICE

I. ANALYSIS OF NON-THYMUS-PROCESSED (B) AND THYMUS-PROCESSED (T) CELL RESPONSES AGAINST METHYLATED BOVINE SERUM ALBUMIN*

BY VOLKER SCHIRRMACHER AND HANS WIGZELL

(From the Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden)

There exist in immunocompetent individuals two major groups of lymphocytes as defined by origin and function (see 1, 2). One group, the non-thymus-processed (B)[1] lymphocytes, have in their lineage the capacity to synthesize humoral antibodies (3). Thymus-processed (T) lymphocytes, on the other hand, have never been found to produce humoral antibodies to any significant degree (4), but can be shown to function as helper cells for B cells when the latter are switched into antibody formation against several types of antigen (5). Both B and T lymphocytes can be shown to carry immunological specificity (6, 7). Whether this is identical or not is still unknown.

Controversy exists as to whether T and B lymphocytes can use the same generator of diversity for creating antigen-reactive cells. According to some investigators there are no detectable immunologlibulin structures of classical type on T cells (8), whereas others report IgM-like molecules on the outer membrane of T lymphocytes (9–12). Making the assumption that B and T lymphocytes use the same genetic system for the creation of antigen-reactive cells, it would seem likely, in view of recent data supporting the germ line theory for explaining antibody diversity (see 13), that the two groups of cells should show similar spectra of reactivity. In accordance with this, studies on cross-reacting albumins suggest that T and B lymphocytes would react with the same structures on the protein antigens (14, 15). On the other hand, using the small peptide hormone glucagon, results obtained showed a region of the molecule as a predominant T cell activator and another site as the epitope against which most B cell products (= humoral antibodies) were directed (16). Specificity differences of immune cells, responding either by DNA synthesis or by antibody production have been demonstrated also through the use of a cellular immunoadsorbent technique (17). Dissimilar patterns of cross-reactivity of humoral antibodies and cellular immune responses have recently been observed by several groups (18–22).

---

[1] *Abbreviations used in this paper:* AFCP, antibody-forming cell precursor; B cell, non-thymus-processed lymphocyte; BSA, bovine serum albumin; BSS, balanced salt solution; CGG, chicken gamma globulin; HSA, human serum albumin; MBSA, MHSA, methylated BSA and HSA; NIP, 4-hydroxy-3-iodo-5-nitrophenylacetic acid; OA, ovalbumin; PVP, polivinylpyrrolidone; sulf, sulfanyl group; T cell, thymus-processed lymphocyte; 1°, 2°, primary and secondary immunization.

We have approached the problem of recognition of antigen by B vs. T lymphocytes by using hapten-carrier systems, allowing the investigator to distinguish between T and B cell activity against the same molecule (1, 23, 24). Bovine serum albumin (BSA) and its methylated derivative (MBSA) were compared for their ability to induce BSA-specific antibody-forming cell precursor (AFCP, B) or helper (T) activity. It has been shown that mice immunized with methylated albumins preferentially develop delayed hypersensitivity, whereas the native albumins induce both Arthus reaction and delayed hypersensitivity (25). Our results demonstrate that while methylation of BSA drastically reduces the B cell response against BSA, the T cell response is unaffected as tested in quantitative assays. The experiments exclude that this is due to a changed likelihood per se of MBSA to make contact with B lymphocytes but would rather suggest different recognition systems for antigen on B and T lymphocytes.

Materials and Methods

Antigens.—The following immunogens were used: bovine serum albumin ([BSA] Cohn fraction V) and chicken gamma globulin ([CGG] Cohn fraction II), both Koch-Light Laboratories Ltd., Colnbrook, Buckinghamshire, England; human serum albumin ([HSA] 4 × crystallized; Nutritional Biochemicals Corporation, Cleveland, Ohio); and ovalbumin ([OA] grade V; Sigma Chemical Company, St. Louis, Mo.).

Methylated BSA (MBSA) or HSA (MHSA) were prepared according to the method described by Fraenkel-Conrat and Olcott (26), which allows a practically complete esterification of the carboxyl groups in the absence of any other esterification. 1 g of albumin, suspended in 100 ml of absolute methanol, was incubated after the addition of 1 ml of concentrated hydrochloric acid for 4 days at room temperature. It was then dialyzed against distilled water and equilibrated with balanced salt solution (BSS). The preparations were subsequently kept frozen at −20°C.

Hapten-carrier conjugates were synthesized using either sulfanilic acid (sulf) or NIP (4-hydroxy-3-iodo-5-nitrophenylacetic acid) as haptenic groups. Coupling reactions and determinations of the number of hapten groups bound per protein molecule were performed as described (24, 27, 28). The conjugates used were: $sulf_8$-BSA, $sulf_5$-OA, $sulf_{12}$-CGG, $sulf_{12}$-MBSA, $sulf_9$-HSA, $sulf_6$-MHSA, NIP_7-BSA, NIP_3-OA, and NIP_9-MBSA.

MBSA and BSA have been compared in vitro with respect to their reactivity with anti-BSA antibodies. In passive hemagglutination inhibition studies the sulf conjugates of BSA and MBSA have been compared as inhibitors, since unconjugated MBSA interfered with the agglutination reaction at concentrations $>10^{-7}$ M because of its positive charge. As can be seen from Fig. 1, sulf-MBSA inhibited the BSA-specific agglutination only at much higher concentrations than sulf-BSA (a factor of 300 at 50% inhibition).

Animals, Immunization, and Cell Transfer.—Adoptive secondary anti-sulf responses were investigated in DBA/2 × C57BL/6 mice. High responsiveness to sulf seems genetically linked to H-2^d (H. Seiler, V. Schirrmacher, unpublished data).[2] CBA mice were used to study anti-NIP responses. Donor mice were primarily immunized with 100 μg of antigen emulsified in complete Freund's adjuvant (Ditco Laboratories, Inc., Detroit, Mich.) and administered subcutaneously into the footpads of the hind legs and in the flanks (total volume 0.2 ml).

---

[2] Rubin, B. 1972. The immune response against hapten-autologous protein conjugates in the mouse. I. Specificity of antibodies produced during the primary response against dinitrophenylated mouse serum albumin. Manuscript submitted for publication.
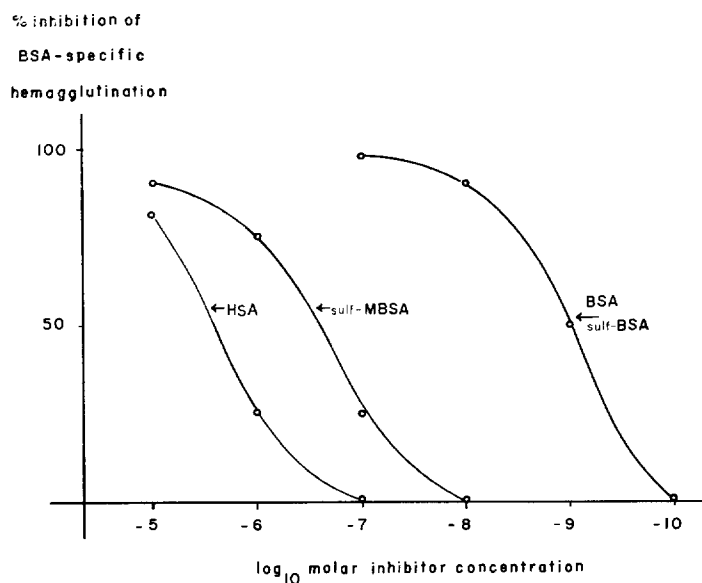
Fig. 1. Inhibition of BSA-specific hemagglutination by BSA, sulf-BSA, sulf-MBSA, and HSA. The anti-BSA antiserum was a pool of sera from DBA/2 × C57BL/6 animals immunized 4 wk before with 100 μg of BSA.

Immune cell suspensions of donor mice were prepared from their spleens and lymph nodes (popliteal, inguinal, and axillary) by pressing them through a stainless steel mesh. Single cell suspensions in BSS were prepared, washed, and counted for number and viability using trypan blue. Varying numbers of viable cells (indicated in each experiment) were transferred together with antigen intravenously into recipient mice of the same strain. These had previously been X-irradiated (29, 30) with 500 R on the same day. 10 days later the mice were bled from the retroorbital plexus and the individual sera titrated for their antibody content.

Serology and Statistical Analyses.—Anti-BSA and anti-sulf antibody titers were determined by passive hemagglutination using sheep erythrocytes coated with BSA by glutaraldehyde[2] or directly coupled with sulf as described previously (31). All hemagglutination titers reported here were resistant to reduction with 0.1 M 2-mercaptoethanol. Anti-BSA titers could be inhibited by BSA at about 10^{-9} M (Fig. 1) and anti-sulf titers by sulfanyl-N-chloracetyl-L-tyrosine (32) at about 10^{-7} M.

Binding capacities were determined for anti-NIP and anti-BSA antisera in a modified Farr ammonium sulfate precipitation assay by using ^{125}I-labeled NIP-ε-aminocaproic acid (aminocap-N ^{125}IP) as hapten (27) or BSA-I^{125} (30). All binding data indicate micrograms of hapten or antigen bound per milliliter of serum. They were calculated from the serum dilution at which 50% of the hapten/antigen in the incubation mixture (0.001 μg/ml aminocap-N^{125}IP or 0.05 μg/ml BSA-^{125}I) was bound.

Serum antibody values were logarithmically transformed and means and standard errors were calculated. In some experiments, determination of the "intragroup" variance was carried out using all data of a given experiment. Use has been made of this variance to calculate the difference (d) that would be significant ($P = 0.05$) in a Student's t test between two of the experimental groups in this experiment.

Experimental Design.—The aim of the present study was to compare the specificities of antigen-specific receptors on AFCP (B) and helper (T) lymphocytes. According to the coop-

eration hypothesis (1, 5, 24), the induction of antibody synthesis against a thymus-dependent antigen requires an interaction of both AFCP and helper cells. It is, therefore, important to establish a system where at least one of these cell types can be tested separately. The adoptive cell transfer system outlined in Fig. 2 was used for this purpose. This allows the helper cells to be tested independently by their capacity to cooperate with hapten-specific AFCP cells in a secondary anti-hapten response.

Immune cells from donor mice immunized against carrier A (BSA) or A' (MBSA) are transferred together with hapten-specific cells from donor mice immunized against hapten-carrier B (sulf-OA or NIP-OA) into a sublethally irradiated host. Stimulation with hapten-
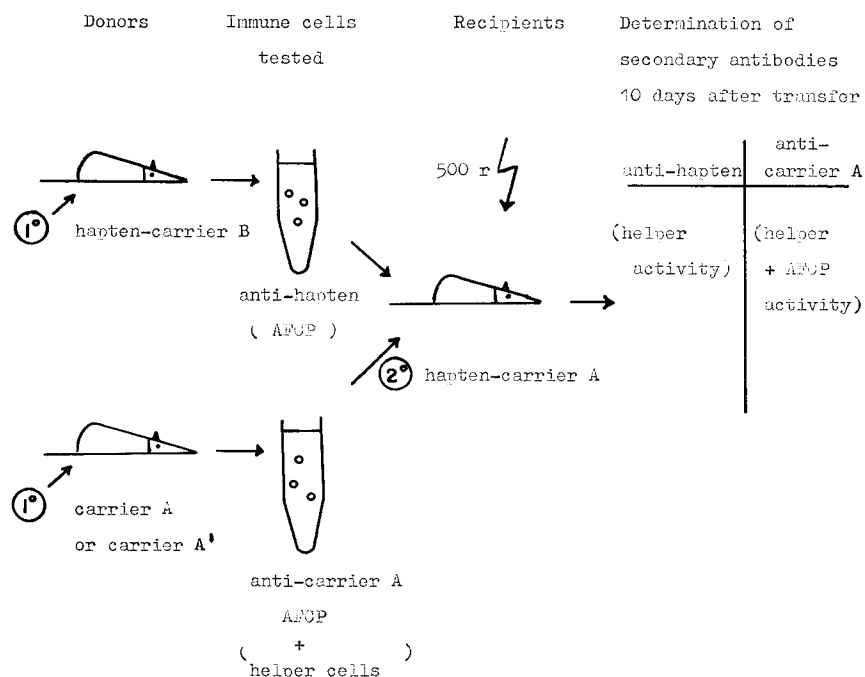

Fig. 2. Experimental design.

carrier A (sulf-BSA or NIP-BSA) induces secondary anti-hapten and anti-carrier A responses. which can be measured 10 days after transfer. By subtracting the relevant control responses (stimulation in the absence of carrier A-specific cells), we determine the helper activity (anti-hapten response) and the helper plus AFCP activity (anti-carrier A response) in the carrier A-specific immune cell population.

In the present study, this system was used to investigate in which way a chemical modification of BSA (methylation) affects its ability to interact with BSA-specific AFCP and helper cells. These studies include (a) comparison of BSA and MBSA immune cells in two cooperative cell transfer systems (anti-NIP and anti-sulf response), (b) transfer of decreasing amounts of BSA or MBSA immune cells in a cooperative cell transfer system, (c) test for the specificity of the helper cells in BSA and MBSA immune cells, (d) comparison of the threshold dose for the induction of BSA-specific helper cells by BSA or MBSA, and (e) comparison of the threshold dose for the cooperative secondary anti-hapten response when hapten-BSA or hapten-MBSA are taken as secondary hapten-carrier conjugates.

RESULTS

Comparison of BSA and MBSA Immune Cells in Cooperative Cell Transfer Systems.—Table I shows the result of an experiment in which 2×10^7 BSA or MBSA immune cells from donor animals immunized 4 wk before were transferred together with NIP-OA immune cells and stimulated with NIP-BSA. Good cooperative anti-NIP responses were obtained in both cases (compare groups III and IV with II). However, when the secondary anti-BSA responses in groups III and IV were compared, a clear-cut reduction in case of transferred MBSA immune cells (group IV) was observed (reduction factor > 20).

In Table II BSA and MBSA immune cells are compared in a cooperative

TABLE I

Comparison of BSA and MBSA Immune Cells. BSA-Specific Helper vs. BSA-Specific Antibody Response in a Cooperative Cell Transfer System (Anti-NIP)

Group No.	Immune cells transferred at day 0*	2° antigen	Antigen-binding capacity at day 10‡	
			Anti-NIP (log₁₀)	Anti-BSA (log₁₀)
		10 µg		
I	NIP-OA	NIP-OA	0.654 ± 0.110	< -1.000
II	"	NIP-BSA	-0.600 ± 0.158	< -1.000
III	" + BSA	"	0.239 ± 0.072	0.731 ± 0.224
IV	" + MBSA	"	0.333 ± 0.096	-0.632 ± 0.126

* 10^7 NIP-OA immune cells (5 months); 2×10^7 BSA or MBSA immune cells (4 wk).

‡ µg aminocap-N^{125}IP or BSA-^{125}I bound per milliliter of serum; mean $\pm$ standard error of log ₁₀ values; six animals per group.

TABLE II

Comparison of BSA and MBSA Immune Cells. BSA-Specific Helper vs. BSA-Specific Antibody Response in a Cooperative Cell Transfer System (Anti-sulf)

Group No.	Immune cells transferred at day 0*	2° antigen	Hemagglutination titer at day 10‡	
			Anti-sulf	Anti-BSA
		50 µg		
I	sulf-OA	sulf-OA	5.2 ± 0.40	0.0
II	"	sulf-BSA	0.2 ± 0.15	0.0
III	"	sulf-MBSA	1.9 ± 0.63	0.0
IV	" + BSA	—	0.3 ± 0.50	3.6 ± 1.22
V	" + "	sulf-BSA	3.3 ± 0.81	9.2 ± 1.20
VI	" + "	sulf-MBSA	4.1 ± 0.38	5.5 ± 0.92
VII	" + MBSA	—	0.8 ± 1.10	0.8 ± 1.33
VIII	" + "	sulf-BSA	3.5 ± 0.51	4.0 ± 1.37
IX	" + "	sulf-MBSA	4.6 ± 0.54	1.9 ± 0.80

* 2×10^7 sulf-OA immune cells (5 wk); 1.5×10^7 BSA and MBSA immune cells (11 days).

‡ Log₂ passive hemagglutination titer; mean $\pm$ one standard deviation; five animals per group.

secondary anti-sulf response. In this experiment BSA and MBSA have been used also as secondary carriers for the sulf hapten (for reactivity of sulf-BSA and sulf-MBSA with anti-BSA antibodies see Fig. 1). The carrier specificity of the adoptive secondary anti-sulf response is shown in the first three groups. Very similar cooperative anti-sulf responses were obtained when BSA or MBSA immune cells were transferred together with sulf-OA immune cells and stimulated either with sulf-BSA (groups V and VIII) or sulf-MBSA (groups VI and IX). Despite this similarity in helper activity, the AFCP activity of the BSA and MBSA immune cells seemed to be quite different. The anti-BSA titers in groups V and VIII differ by about five log$_2$ units. A reduction of secondary anti-BSA titers can be seen also between groups V and VI and between groups VIII and IX.

In order to obtain more detailed information about helper and AFCP activity in BSA and MBSA immune cell suspensions, limiting dilution assays were carried out in two further experiments listed in Table III. In experiment No. 1, 5 and 1 million BSA immune cells were somewhat better as helpers than MBSA immune cells; in experiment No. 2, MBSA immune cells were better as helpers throughout all doses tested. Taking both experiments together there appears to be no clear difference between BSA-specific helper activity in BSA and MBSA

TABLE III

Comparison of BSA and MBSA Immune Cells. Transfer of Decreasing Amounts of Cells in a Cooperative Cell Transfer System

Experiment	Group No.	Immune cells transferred at day 0*	2° antigen	Hemagglutination titer at day 10‡	
				Anti-sulf	Anti-BSA
			50 µg		
1	I	sulf-OA	sulf-BSA	1.5	0.0
	II, III	" + 25 BSA (MBSA)	"	8.0 (8.1)	7.1 (1.9)
	IV, V	" + 5 " (")	"	6.6 (5.4)	2.5 (0.0)
	VI, VII	" + 1 " (")	"	3.9 (2.6)	0.0 (0.0)
	VIII, IX	" + 0.2 " (")	"	2.0 (2.1)	0.0 (0.0)
2	I	sulf-OA	sulf-OA	6.4	0.0
	II	"	sulf-BSA	0.4	0.0
	III, IV	" + 25 BSA (MBSA)	"	3.8 (6.1)	11.3 (10.0)
	V, VI	" + 5 " (")	"	2.5 (3.4)	8.5 (3.3)
	VII, VIII	" + 1 " (")	"	0.7 (1.7)	4.7 (0.8)

* Donor cells in experiment 1: 4 × 10^7 sulf-OA immune cells (3 months); BSA and MBSA immune cells (amounts transferred indicated in millions) (14 days). Donor cells in experiment 2: 2 × 10^7 sulf-OA immune cells (8 wk); BSA and MBSA immune cells (amounts transferred indicated in millions) (4 wk). The responses obtained with MBSA immune cells are given in parentheses.

‡ Log$_2$ passive hemagglutination titer; mean values of five animals per group. Smallest significant difference (P = 0.05) for experiment 1: d = 1.2, for experiment 2: d = 1.5.

immune cell suspensions. On the other hand, a pronounced reduction of anti-BSA titers in case of transferred MBSA immune cells was again observed in both experiments (compare groups II and III in experiment 1 and V and VI in experiment 2).

The experiments reported so far do not answer the question of whether MBSA induces specific helper cells or exerts its activation of helper function in a nonspecific way, e.g., due to its positive charge it might perhaps induce cells to produce a factor substituting for carrier-specific helper cells. The helper specificity of BSA and MBSA immune cells was investigated (Table IV). The anti-sulf responses in groups VI–IX demonstrate that MBSA as well as BSA immune cells have a helper activity specific for BSA but not HSA (for cross-reactivity between BSA and HSA see Fig. 1). When MBSA and MHSA were taken as secondary carriers (groups X–XIII) a cooperative anti-sulf response was obtained only with the former. Apart from confirming the BSA specificity of the helper cells, these results indicate that the methylation procedure has not introduced into the albumin molecules new determinants which cross-react on the helper level. On the antibody level no new determinants could be detected either (25).

Threshold Dose for the Activation of BSA-Specific Helper Cells by BSA or MBSA.—The comparison of BSA and MBSA immune cells showed that MBSA had a reduced ability to react with BSA-specific AFCP cells, whereas there was no reduction of its ability to react with BSA-specific helper cells. One could argue that in the experiments reported the antigen doses were too high to allow a detection of a difference between the reactivity of BSA and MBSA at the helper level. Therefore the threshold doses for the activation of

TABLE IV

Comparison of BSA and MBSA Immune Cells. Specificity of the Helper Cells.

Group No.	Immune cells transferred at day 0*	2° antigen	Hemagglutination titer at day 10‡	
			Anti-sulf	Anti-BSA
		20 μg		
I	sulf-CGG	sulf-CGG	5.4	0.0
II	"	sulf-BSA	1.4	0.0
III	"	sulf-HSA	1.2	0.0
IV	"	sulf-MBSA	1.8	0.0
V	"	sulf-MHSA	1.9	0.0
VI, VII	" + BSA (MBSA)	sulf-BSA	4.5 (3.5)	9.3 (3.7)
VIII, IX	" + " (")	sulf-HSA	1.4 (1.3)	3.5 (0.0)
X, XI	" + " (")	sulf-MBSA	3.7 (3.7)	5.5 (0.5)
XII, XIII	" + " (")	sulf-MHSA	1.6 (1.9)	1.7 (0.1)

* 1.5 × 10^7 sulf-CGG immune cells (4 months); 2 × 10^7 BSA or MBSA immune cells (17 days). The responses obtained with MBSA immune cells are given in parentheses.

‡ Log_2 passive hemagglutination titer; mean of five animals per group. Smallest significant difference ($P = 0.05$) $d = 1.3$.

BSA-specific helper cells by BSA or MBSA were determined: (a) for primary induction of helper cells (Table V) and (b) for reactivity with the helper cells in a cooperative secondary anti-hapten response (Table VI).

Table V shows the results of an experiment where BSA and MBSA immune cells from animals immunized with different antigen doses (100–0.1 µg) were transferred together with sulf-OA immune cells and tested for their helper

TABLE V

Threshold Dose for the Acitvation of BSA-Specific Helper Cells by BSA or MBSA. Primary Induction of Helper Cells

Group No.	Immune cells transferred at day 0*		2° antigen	Hemagglutination titer at day 10‡	
				Anti-sulf	Anti-BSA
		50 µg			
I	sulf-OA		sulf-OA	8.4	0.0
II	"		sulf-BSA	1.9	0.0
III, IV	"	+ BSA (MBSA) 1° 100	"	8.0 (6.2)	5.8 (0.6)
V, VI	"	+ " (") 1° 10	"	8.9 (7.3)	5.2 (0.8)
VII, VIII	"	+ " (") 1° 1	"	5.5 (4.3)	0.9 (0.0)
IX, X	"	+ " (") 1° 0.1	"	2.5 (2.0)	0.0 (0.0)
XI	"	+ normal cells (FC)	"	1.8	0.0

* 3 × 10⁷ sulf-OA immune cells (2 months); 2 × 10⁷ BSA or MBSA immune cells from animals immunized 2 wk before with the indicated amount (µg) of antigen in Freund's complete adjuvant (FC). Control (XI): cells from animals immunized 2 wk before with FC alone. The responses obtained with MBSA immune cells are given in parentheses.

‡ Log_2 passive hemagglutination titer; mean of five animals per group. Smallest significant difference ($P = 0.05$) $d = 1.2$.

TABLE VI

Threshold Dose for the Activation of BSA-Specific Helper Cells by BSA or MBSA. Induction of a Secondary Anti-NIP Response with BSA-Specific Helper Cells and NIP-BSA or NIP-MBSA as Antigen

Group No.	Immune cells transferred at day 0*		2° antigen	Dose	Anti-NIP at day 10‡ (log_{10})
				µg	
I	NIP-OA		NIP-OA	10	0.348 ± 0.189
II	"		NIP-BSA	10	0.039 ± 0.057
III	"		NIP-MBSA	10	0.042 ± 0.061
IV	"	+ BSA	NIP-BSA	1	0.012 ± 0.103
V	"	+ "	"	10	0.427 ± 0.126
VI	"	+ "	NIP-MBSA	1	0.013 ± 0.073
VII	"	+ "	"	10	0.306 ± 0.086

* 1.6 × 10⁷ NIP-OA immune cells (3 months); 2 × 10⁷ BSA immune cells (6 wk).

‡ µg aminocap-N¹²⁵IP bound per milliliter of serum; mean ± standard error of log_{10} values; six animals per group.

activity in a secondary anti-sulf response. Cells from animals immunized with complete Freund's adjuvant alone (group XI) and from animals immunized with 0.1 μg of BSA or MBSA (groups IX and X) contained no helper activity. By increasing the antigen dose, a helper activity was demonstrated in both cases (BSA and MBSA) at a dose of 1 μg per animal (groups VII and VIII). This indicates that there is no significant difference in the threshold dose for the induction of BSA-specific helper cells by BSA or MBSA.

The threshold dose of BSA and MBSA as secondary carriers in a cooperative secondary anti-NIP response with BSA-specific helper cells was determined in the experiment shown in Table VI. NIP-BSA and NIP-MBSA did not induce a cooperative anti-NIP response at a dose of 1 μg (groups IV and VI) but did so at 10 μg (groups V and VII). The latter responses were as high as the homologous response (group I). This demonstrates that the ability of BSA to react with BSA-specific helper cells was not affected by the methylation procedure. This finding was confirmed in the secondary anti-sulf response. Sulf-OA immune cells were transferred alone or together with BSA immune cells into recipient mice which were stimulated with different amounts of either sulf-BSA or sulf-MBSA. As shown in Fig. 3 the dose-response curves were rather similar, the one obtained with sulf-MBSA being shifted to lower antigen concentrations. The latter observation could be explained by a higher intrinsic immunogenicity of sulf-MBSA compared with sulf-BSA, since it induced a higher anti-sulf response also in the absence of BSA-specific helper cells (compare the heterologous responses in Fig. 3). Thus, in none of the systems tested did BSA-specific helper cells react less well with MBSA than with BSA.

DISCUSSION

In the present article we have presented evidence that methylation of BSA to more than 95% reduces its ability to react with anti-BSA antibodies (Fig. 1) and with BSA-specific AFCP (B) cells. However, its reactivity with BSA-specific helper (T) cells remains unchanged, as demonstrated by an identical threshold concentration of BSA and MBSA for activation of helper cells. The differential effect of methylation could be shown on two levels: (a) for the primary induction of BSA-specific AFCP or helper cells by BSA and MBSA, respectively, and (b) for the secondary interaction of BSA-specific AFCP or helper cells with hapten-BSA and hapten-MBSA conjugates.

The two main entities upon which valid interpretation of our data depend are the types of immune cells involved in the cooperative cell transfer system studied and the impact of the methylation of BSA on its physicochemical properties, which could affect its recognition by AFCP and helper cells.

The experimental evidence identifying helper cells as T and AFCP as B cells may be summarized as follows: (a) Educated thymus cells substitute for carrier-specific helper cells (1, 33), whereas anti-carrier antibody does not, at least in conventional hapten-carrier systems (1, 24). (b) Anti-θ treatment in the presence of complement inhibits the function of carrier-specific helpers but not

of hapten-specific AFCP cells (34). (c) Helper cells are not retained on anti-immunoglobulin-coated columns, which do retain AFCP lymphocytes (35, 36). (d) Lymphocytes potentially capable of producing measurable humoral antibody belong to the B type only (4).

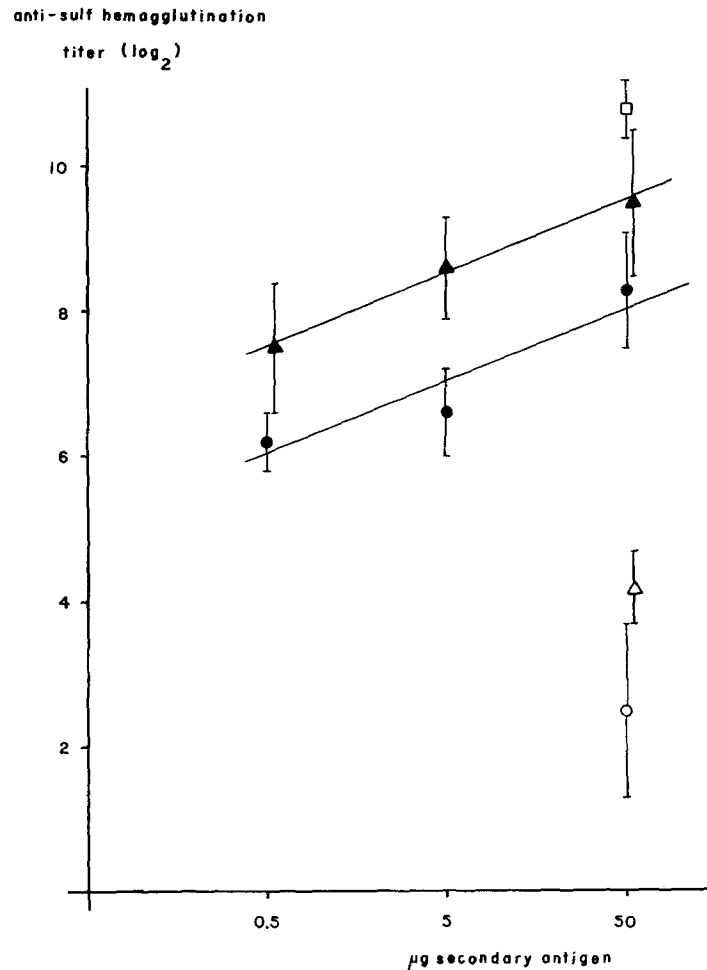With these properties of helper (T) and AFCP (B) cells in mind we may ex-

Fig. 3. Comparison of cooperative anti-sulf responses obtained with sulf-BSA and sulf-MBSA in an adoptive cell transfer system. 500 R irradiated mice were injected with 3×10^7 sulf-OA immune cells (8 wk) alone (open symbols) or together with 1.5×10^7 BSA immune cells (8 wk, closed symbols). They were stimulated with the indicated amounts of sulf-OA (□), sulf-BSA (●, ○), or sulf-MBSA (▲, △). Symbols and vertical bars represent geometric means and standard deviations of anti-sulf hemagglutination titers ($\log_2$) obtained 10 days after cell transfer (six animals per group). Anti-sulf titers were titrated in phosphate-buffered saline containing 0.45% PVP K 60 (Alex Engblom, Borås, Sweden), which increased the sensitivity of the assay by three $\log_2$ titers (V. Schirrmacher, unpublished data).

amine the impact of the methylation of BSA on its physicochemical properties which could influence cellular recognition. Since the methylation reaction involves only the negatively charged carboxyl groups of the molecule (26), the most pronounced effect of the chemical modification is an alteration of charge. In agarose electrophoresis at pH 7.2 BSA moves to the positive, MBSA to the negative electrode, whereas sulf-MBSA is neutral.[3] One could therefore try to attribute the differential reactivity of BSA-specific AFCP and helper cells with MBSA to its altered charge. There are two arguments against this assumption: (a) the differential reactivity of BSA-specific B and T cells with MBSA is also observed when MBSA is presented as a neutral sulf-MBSA conjugate, and (b) chemical modifications at the positively charged amino groups of BSA (e.g. acetylation) had a similar effect as the methylation.[3]

An alternative explanation for the poor humoral anti-BSA response induced by MBSA would be that MBSA as a consequence of a change in physicochemical properties is less likely in vivo to interact with any kind of B lymphocytes than native BSA. Arguments against this interpretation come from the results obtained when having the hapten on BSA or MBSA as secondary carriers in cooperative cell transfer systems using graded doses of antigen for secondary stimulation. Here, the anti-hapten response would require the hapten-carrier complex to come in close physical contact with the B lymphocyte immune to the hapten determinant. The results demonstrate no difference between hapten-BSA and hapten-MBSA conjugates in such tests, thus excluding the possibility that MBSA is in itself different from BSA with regard to making physical contact to B lymphocytes.

Having so far argued against unspecific factors which could have influenced our results, we must consider the possibility that B and T lymphocytes may have different or largely nonoverlapping spectra of inducing antigenic specificities. Glucagon has been shown to carry on its carboxyl end a T cell-inducing site whereas humoral antibody is directed primarily against epitopes at the amino-terminal piece (16). Recent data from other groups (21, 19) support our observations with methylated BSA, in so far as they also demonstrate a differential effect of chemically modified proteins (i.e. acetoacetylated flagellin and reduced S-carboxymethylated lysozyme) on their ability to induce humoral and cell-mediated immune responses. It is tempting to speculate on the fact that all these modifications exert their effect in the same direction, namely suppression on the AFCP level. This seems to hold true also for complex cellular antigens such as sheep red blood cells (22), an observation which hopefully will have practical implications, e.g., in tumor therapy. In some systems the suppressive effect on the antibody level is accompanied by an even enhanced ability of the modified antigen to induce cell-mediated immunity (21, 22). These data in combination with our results would suggest that T cells can recognize

---

[3] Schirrmacher, V., and H. Wigzell. Immune responses against native and chemically modified albumins in mice. II. Manuscript in preparation.

other structures on a macromolecule than can B cells (with some structures recognized by both), whereas work on cross-reacting albumins has yielded results suggesting similarity if not identity between T and B cell receptor specificities (14, 15).

At this point it should be realized that recognition with regard to T or B cell activation by antigen involves at least two steps, actual specific combination between antigen-binding receptors on the cell surface with antigen plus a relatively nonspecific activation process characteristic for the cell type involved (37). That such a latter switch-on process might be highly typical for one of the two groups of lymphocytes is shown by studies on mitogens, where the mitogen can be shown to be bound equally well (e.g. phytohemagglutinin or concanavalin A [38]) to both B and T lymphocytes. Yet, in the soluble form of the mitogen, only T cell activation will take place, whereas if the same mitogen is rendered insoluble primarily B cell activation will be seen (39). Thus, analogies between binding of antigen to lymphocytes as a direct measure of activation might be very misleading.

However, some interpretations of the present results are possible with regard to antigen-binding receptors on B vs. T cells. MBSA will combine 300 times less well than BSA with anti-BSA antibodies (Fig. 1), suggesting that 0.3% of native determinants are left on MBSA. Humoral antibodies can be considered as good analogues of soluble antigen-binding receptors for at least B lymphocytes (40) and humoral antibodies should have, if anything, a better access to antigenic sites than would cell-bound receptors for antigen. The finding that, despite this, MBSA is equal to BSA with regard to inducing anti-BSA helper activity when using graded doses of antigen in primary or secondary immune systems, we consider incompatible with the idea that BSA-specific B and T cells react with the very same determinants.

Differences in specificities of B and T cells, as they are now apparent in several systems (16–22, 41–44), could mean either (a) that the recognition systems for antigen on B and T cells are principally different or (b) that they are of identical nature, only differing in the spectra of their specificities. The latter could have been affected by genetically determined unresponsiveness (the defect appears to localize preferentially either in AFCP or helper cells [see 45]) and/or by different selection mechanisms operating during ontogeny in the diversification of B and T cell specificity (46, 47). We would favor conception a since it allows the assumption that T cells recognize larger areas on a protein molecule than do B cells. This would be a reasonable way of explaining why chemical modifications of antigens preferentially suppress humoral antibody induction against it.

SUMMARY

Immune cells induced by bovine serum albumin (BSA) and its methylated derivative (MBSA) have been compared in a cooperative cell transfer system

for their content of BSA-specific antibody-forming cell precursors (AFCP, B) and BSA-specific helper (T) cells. When MBSA immune cells were transferred together with hapten-primed cells into recipient mice which were stimulated by a hapten-BSA conjugate, their cooperative secondary anti-hapten response was as good as in case of transferred BSA immune cells. Their secondary anti-BSA response, however, was markedly reduced (reduction factor > 30). Hapten-MBSA conjugates had the same capacity to react with BSA-specific helper cells in the cooperative secondary anti-hapten response as hapten-BSA conjugates but had a reduced ability to react with BSA-specific AFCP cells. In spite of the pronounced reduction of the B cell response, MBSA had the same threshold dose as BSA for activating BSA-specific T cells.

These data suggest that B and T cells recognize different epitopes on the BSA molecule, only those recognized by B cells being affected by the methylation procedure.

REFERENCES

1. Mitchison, N. A., K. Rajewsky, and R. B. Taylor. 1970. Cooperation of antigenic determinants and of cells in the induction of antibodies. *In* Developmental Aspects of Antibody Formation and Structure. J. Šterzl, editor. Academia, Publishing House of the Czechoslovak Academy of Sciences, Praha. 547.

2. Miller, J. F. A. P., A. Basten, J. Sprent, and C. Cheers. 1971. Interaction between lymphocytes in immune responses. *Cell. Immunol.* **2:**469.

3. Mitchell, G. F., and J. F. A. P. Miller. 1968. Cell to cell interaction in the immune response. II. The source of hemolysin-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. *J. Exp. Med.* **128:**821.

4. Davies, A. J. S., E. Leuchars, V. Wallis, R. Marchant, and E. C. Elliott. 1967. The failure of thymus-derived cells to produce antibody. *Transplantation.* **5:**222

5. Mitchison, N. A. 1971. The carrier effect in the secondary response to hapten-protein conjugates. II. Cellular cooperation. *Eur. J. Immunol.* **1:**18.

6. Basten, A., J. F. A. P. Miller, N. L. Warner, and J. Pye. 1971. Specific inactivation of thymus-derived (T) and non-thymus-derived (B) lymphocytes by ^{125}I-labelled antigen. *Nat. New Biol.* **231:**104.

7. Miller, J. F. A. P., and J. Sprent. 1971. Cell-to-cell interaction in the immune response. VI. Contribution of thymus-derived cells and antibody forming cell precursors to immunological memory. *J. Exp. Med.* **134:**66.

8. Crone, M., C. Koch, and M. Simonsen. 1972. The elusive T cell receptor. *Transplant. Rev.* **10:**36.

9. Mason, S., and N. L. Warner. 1970. The immunoglobulin nature of the antigen recognition site on cells mediating transplantation immunity and delayed hypersensitivity. *J. Immunol.* **104:**762.

10. Hogg, N. M., and M. F. Greaves. 1972. Antigen-binding thymus-derived lymphocytes. II. Nature of immunoglobulin determinants. *Immunology.* **22:**967.

11. Hämmerling, U., and K. Rajewsky. 1971. Evidence for surface associated immunoglobulins on T and B lymphocytes. *Eur. J. Immunol.* **1**:447.

12. Marchalonis, J. J., R. E. Cone, and J. L. Atwell. 1972. Isolation and partial characterization of lymphocyte surface immunoglobulins. *J. Exp. Med.* **135**:956.

13. Second IUIS Symposium on Genes and Antibodies. 1972. *Scand J. Immunol.* **1**:283.

14. Rajewsky, K., and H. Pohlit. 1971. Specificity of helper function. *Prog. Immunol.* **1**:337.

15. Rajewsky K., C. Brenig, and I. Melchers. 1971. Specificity and suppression in the helper system. *In* Cell Interactions. Le Petit Symposium. L. G. Silvestri, editor. North Holland Publishing Company, Amsterdam. **3**.

16. Senyk, G., E. B. Williams, D. E. Nitecki, and J. W. Goodman. 1971. The functional dissection of an antigen molecule: specificity of humoral and cellular immune responses to glucagon. *J. Exp. Med.* **133**:1294.

17. Davie, J. M., and W. E. Paul. 1970. Receptors on immunocompetent cells. I. Receptor specificity of cells participating in a cellular immune response. *Cell. Immunol.* **1**:404.

18. Cooper, M. G., and G. L. Ada. 1972. Delayed-type hypersensitivity in the mouse. III. Inactivation of thymus-derived effector cells and their precursors. *Scand. J. Immunol.* In press.

19. Thompson, K., M. Harris, and E. Benjamini. 1972. Cellular and humoral immunity: a distinction in antigenic recognition. *Nat. New Biol.* **238**:20.

20. Maron, E., C. Webb, D. Teitelbaum, and R. Arnon. 1972. Cell-mediated vs. humoral response in the cross-reaction between hen egg-white lysozyme and bovine α-lactalbumin. *Eur. J. Immunol.* **2**:294.

21. Parish, C. R. 1971. Immune response to chemically modified flagellin. II. Evidence for a fundamental relationship between humoral and cell-mediated immunity. *J. Exp. Med.* **134**:21.

22. Parish, C. R. 1972. Preferential induction of cell-mediated immunity by chemically modified sheep erythrocytes. *Eur. J. Immunol.* **2**:143.

23. Mitchison, N. A. 1967. Antigen recognition responsible for the induction in vitro of the secondary response. *Cold Spring Harbor Symp. Quant. Biol.* **32**:431.

24. Rajewsky, K., V. Schirrmacher, S. Nase, and N. K. Jerne. 1969. The requirement of more than one antigenic determinant for immunogenicity. *J. Exp. Med.* **129**:1131.

25. Crowle, A. J., C. C. Hu, and A. Patrucco. 1968. Preferential development by mice of delayed hypersensitivity to purified basic proteins. *J. Allergy.* **42**:140.

26. Fraenkel-Conrat, H., and H. S. Olcott. 1945. Esterification of proteins with alcohols of low molecular weight. *J. Biol. Chem.* **161**:259.

27. Brownstone, A., N. A. Mitchison, and R. Pitt-Rivers. 1966. Chemical and serological studies with an iodine-containing synthetic immunological determinant 4-hydroxy-3-iodo-5-nitrophenylacetic acid (NIP) and related compounds. *Immunology.* **10**:465.

28. Tabachnik, M., and H. Sobotka. 1960. Azoproteins. II. A spectrophotometric study of the coupling of diazotized arsanilic acid with proteins. *J. Biol. Chem.* **235**:1051.

29. Mäkelä, O., and N. A. Mitchison. 1965. The role of cell number and source in adoptive immunity. *Immunology.* **8:**539.

30. Celada, F. 1966. Quantitative studies of the adoptive immunological memory in mice. I. An age-dependent barrier to syngeneic transplantation. *J. Exp. Med.* **124:**1.

31. Schirrmacher, V., and K. Rajewsky. 1970. Determination of antibody class in a system of cooperating antigenic determinants. *J. Exp. Med.* **132:**1019.

32. Schirrmacher, V. 1972. The synthesis of radioactively labeled sulfanyl-*N*-chloracetyl-L-tyrosine and its use for determinations of quantities and affinities of anti-*p*-azobenzene-sulfonate antibodies. *Eur. J. Immunol.* In press.

33. Cheers, C., J. Breitner, M. Little, and J. F. A. P. Miller. 1972. *Nature (Lond.).* In Press.

34. Raff, M. C. 1970. Role of thymus-derived lymphocytes in the secondary humoral immune response in mice. *Nature (Lond.).* **226:**1257.

35. Wigzell, H., C. Huber, and V. Schirrmacher. 1971. Affinity fractionation of lymphoid cells according to type and function. International Quarterly of Haematology. In press.

36. Wigzell, H., G. Sundquist, and T. O. Yoshida. 1972. Separation of cells according to surface antigens by the use of antibody-coated columns. Fractionation of cells carrying immunoglobulins and blood group antigens. *Scand. J. Immunol.* **1:**75.

37. Andersson, J. 1972. Mitogens as probes for immunocyte activation. Doctoral Thesis, Stockholm University.

38. Greaves, M. F., S. Bauminger, and G. Janossy. 1972. *Clin. Exp. Immunol.* In press.

39. Andersson, J., G. M. Edelman, G. Möller, and O. Sjöberg. 1972. Activation of B lymphocytes by locally concentrated concanavalin A. *Eur. J. Immunol.* **2:**233.

40. 1970. *Transplant. Rev.* **5**.

41. Stupp, Y., W. E. Paul, and B. Benacerraf. 1971. Structural control of immunogenicity. III. Preparation for and elicitation of anamnestic responses by oligo- and poly-lysines and their DNP derivatives. *Immunology.* **21:**595.

42. Alkan, S. S., D. E. Nitecki, and J. W. Goodman. 1972. Antigen recognition and the immune response. Humoral and cellular immune responses to small mono- and bifunctional antigen molecules. *J. Exp. Med.* **135:**1228.

43. Paul, W. E., D. H. Katz, E. A. Goidl, and B. Benacerraf. 1970. Carrier function in anti-hapten immune responses. II. Specific properties of carrier cells capable of enhancing anti-hapten antibody responses. *J. Exp. Med.* **132:**283.

44. Rubin, B., V. Schirrmacher, and H. Wigzell. 1972. Induction of anti-hapten antibody responses against haptens conjugated to autologous and heterologous proteins. IV. International Congress on Lymphatic Tissues and Germinal Centers. Plenum Publishing Corporation, New York. In press.

45. McDevitt, H. O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Adv. Immunol.* **11:**31.

46. Paul. W. E. 1970. Functional specificity of antigen-binding receptors of lymphocytes. *Transplant. Rev.* **5:**130.

47. Rajewsky, K. 1971. The carrier effect and cellular cooperation in the induction of antibodies. *Proc. R. Soc. Lond. B. Biol. Sci.* **176:**385.