The University of Manitoba

ANTI-IgM SUPPRESSION IN B6AF$_1$ MICE

by

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A Thesis Submitted to the
Faculty of Graduate Studies
in Partial Fulfillment of
the Requirements for the
Degree of Master of Science.

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May, 1976.

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ACKNOWLEDGEMENTS

I am deeply indebted to Dr. E. Sabbadini who gave freely of his time to give his unfailing guidance and support. Special thanks are due to all my collegues who advised and supported me in my studies. I would also like to thank Dr. B. Carter for his generous gift of BALB/C plasma myelomas and Dr. S. Fujimoto for his gift of rabbit anti mouse $F(ab')_2$ serum. The preparation of the photographs of the electrophoresis slides by Mr. H. Sanderson was greatly appreciated. I would also like to thank Ms. A. Pimlott for her extreme patience in typing this manuscript. Finally, I would like to thank my husband for his encouragment and understanding.

ABSTRACT

The objective of the present investigation was to produce a state of agammaglobulinemia in $B6AF_1$ mice with chronic administration of anti μ serum from birth in order to examine T cell mediated reactions in the absence of B cells or the immunoglobulin that they produce. The anti μ treatment resulted in a complete suppression of serum IgM and an incomplete suppression of IgG as shown by immunoelectrophoresis and immunodiffusion. Moreover, there was a complete suppression of the production of IgM antibody to SRBC detected with the direct plaque forming cell assay. An attempt was made to measure the numbers of B cells in the peripheral lymphoid organs of the suppressed mice with anti Ig (RAMIg) serum in a ^{51}Cr release cytotoxic assay. However, the anti Ig serum was unable to quantitate B cells because the serum contained antibody to mouse thymocytes and the absorbed serum did not lyse significant numbers of Ig bearing cells in the presence of complement. AKR anti C_3H θ serum (cytotoxic assay) demonstrated a statistically significant 10% increase in T cells in the lymph nodes of anti μ treated mice over their littermate controls which may indirectly reflect a corresponding B cell loss. Thus, from these results it was concluded that the anti μ suppressed $B6AF_1$ mouse may not be a good model for the study of the regulation by B cells of T cell mediated immune reactions because of the incomplete suppression of B cell activity.

ABBREVIATIONS

PFC	=	Plaque forming cell
B cell	=	Thymus independent lymphocyte
T	=	Thymus dependent lymphocyte
SRBC	=	Sheep red blood cells
PBS	=	Phosphate Buffered Saline
SAS/2	=	Saturated Ammonium Sulphate 50%
BBS	=	Borate Buffered Saline
FCA	=	Freund's Complete Adjuvant
GPC	=	Guinea Pig Complement
RAMLS	=	Rabbit Anti Mouse Lymphocyte serum
OD	=	Optical Density
$(NH_4)_2SO_4$	=	Ammonium Sulphate
ATXBM	=	Adult Thymectomized and Bone Marrow Reconstituted
poly RAMIg	=	Polyvalent Rabbit Anti Mouse Immunoglobulin
RFC	=	Rosette forming cell
ABC	=	Ag binding cells
DH	=	Delayed hypersensitivity
GVH	=	Graft versus host
MLR	=	Mixed lymphocyte reaction
FγG	=	Fowl gamma globulin
β-2-M	=	β-2-microglobulin
DMSO	=	Dimethylsulfoxide
FCS	=	Fetal calf serum
NMS	=	Normal mouse serum

ABBREVIATIONS - cont'd

NH_4Cl	=	Ammonium chloride
GPC	=	Guinea pig complement
NRS	=	Normal rabbit serum
AFC	=	Ab forming cell
H-H	=	Hanks'-Hepes
ADLC	=	Ab dependent lymphocyte mediated cytotoxicity
DLC	=	Direct lymphocyte cytotoxicity
DNP_{34} CSA	=	Dinitrophenylated chicken serum albumin

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ANTI IgM SUPPRESSION IN

$B6AF_1$ MICE

LITERATURE REVIEW

A. Introduction:

The mechanisms involved in the induction and regulation of the immune response are not yet fully understood. It is generally agreed that for the induction of the response, antigen (Ag) must meet some kind of receptor either free in the body fluids or attached to some cell surface. Studies with radioactively labelled antigens (1,2) have shown that only a small proportion of the receptor bearing lymphocytes have the ability to recognize and bind specific antigen *in vitro*.

There has been much evidence establishing the immunoglobulin (Ig) nature of these receptors on the surface of thymus independent B lymphocytes of mice (3-11). The nature of the receptor on the thymus dependent T lymphocytes is still unknown but it is likely that either these cells do not possess any surface Ig or they do so in a very low concentration. Thus, it was expected that the treatment of newborn mice with anti Ig serum would affect only the B lymphocytes. Several studies (12,14,15) have shown that *in vivo* injection of anti Ig antibodies can result in suppressed B cell Ig and Ab formation. However, this suppression is difficult to achieve in the adult animal because it's immunologically competent lymphocytes produce circulating Ig's which prevent the injected anti Ig antibodies from reaching the B lymphocyte. Thus, the injection of anti IgM antibodies into newborn mice which have immunologically incompetent lymphocyte and low serum Ig levels (placental transfer of maternal Ig) will

result in a strong immunosuppression and in some cases result in complete agammaglobulinemia (16,17,19). The objective of the present investigation was to reproduce this immunosuppression in order to examine T cell function in the absence of B cells or the Ig that they produce.

B. Anti IgM Suppression in Mice:

Many studies have been done on the suppressive effects of anti Ig serum on various immunological reactions. The most extensive studies have involved the suppression of antibody synthesis *in vivo* and *in vitro* by anti Ig serum. Anti IgM heavy chain (anti μ) serum has been the most powerful reagent suppressing all the humoral classes of Ab in the mouse strains, BALB/C (15), conventional Swiss mice (12) and nude mice (16,17). Lawton and Cooper (19) also found that in chickens, anti IgM treatment *in ovo* followed by bursectomy resulted in agammaglobulinemic chickens.

Many researchers have worked with anti IgM suppression with generally concordant results. Lawton et al. (15) subjected neonatal BALB/C mice to a series of intraperitoneal injections of purified goat anti mouse IgM serum. Specific anti IgM antibody (0.5 mg) or nonspecific globulin in 0.05 mls of PBS were given on each of the first four days after birth and 1 mg each week thereafter. Quantitation of serum Ig levels in anti u treated mice did not reveal any serum IgM although the sera were found to contain goat antibodies to mouse IgM. The suppressed mice showed significantly lowered concentrations of serum IgG ($P<0.05$), IgG_2 ($P<0.025$) and IgA ($P<0.005$) from littermate controls. Manning (14) administered a similar series of intraperitoneal injections of rabbit anti μ chain serum into newborn BALB/C mice. Injections were administered on the day of

birth and subsequent doses were given at 2-7 day intervals. Ouchterlony analysis of Ig levels in the young adult revealed a complete absence of serum IgM and a reduction of other serum immunoglobulins (IgG, IgA) to low residual levels. However, if the treatment was ceased after the first fifteen days of life and the animals were allowed to coast until day 46, serum IgM and IgG levels were seen to recover to control values.

Nude mice were found to be much more sensitive to anti μ treatment than normal littermates (17). Athymic BALB/C mice were given 0.05 mls of purified rabbit anti u globulin on day 0 and increasing doses on alternate days until a maximum of 0.20 mls of globulin was achieved on day 30. Alternate day injections of this maximum dose (0.20 mls) was continued through day 56. All of the nude mice given this suppressive treatment demonstrated an absolute loss of IgM and IgA. Serum levels of IgG_1 and IgG_2 were shown to exhibit considerable variation between litters (see Fig.I).

Manning (16) examined the suppressive effects of the dose and the injection schedule of the anti μ treatment on Ig levels in nude mice and littermate BALB/C mice (see Fig.II). The pattern of suppression induced by a slight suppressive schedule (0.47 mls in first 15 days) was similar in both groups, i.e. complete suppression of IgM and IgA and severe reduction of IgG_1 and IgG_2. However, by day 46, the littermates demonstrated considerable recovery of both IgG subclasses as well as IgM and IgA. An increased suppressive schedule (3.15 mls over 42 days) resulted in suppressed IgM and IgA levels which showed no recovery at 47 days. The suppression of the IgG classes was also maintained at 47 days.

The ability of both nude mice and their littermates to recover

Fig.I. (17) Individual Immunoglobulin and Antibody Levels in Nude Mice Injected with Anti-Ig Antisera

Mouse No. (Litter no.)	Injection	Ig or antibody levels[c] at Day 32/Day 57					
		IgM	IgG1	IgG2	IgA	Anti-rabbit	Anti-Ig
1 (1)	Anti-μ	0/0	8/16	8/8	0/0	0/0	16/16
2 (1)	Anti-μ	0/0	8/16	8/8	0/0	0/0	16/16
3 (1)	Anti-μ	0/0	8/16	8/8	0/0	0/0	16/16
4 (2)	Anti-μ	0/0	2/0	0/0	0/0	0/0	32/32
5 (2)	Anti-μ	0/0	2/8	0/0	0/0	0/0	32/32
6 (3)	Anti-μ	0/0	2/0	0/0	0/0	0/0	32/16
7 (4)	Anti-α	32/32	8/4	32/256	0/0	0/0	16/8
8 (5)	Anti-α	32/32	4/1	32/128	0/0	0/0	16/8
9 (6)	Anti-α	16/32	1/2	32/64	0/0	0/0	8/8
10 (6)	Anti-α	32/32	2/16	64/128	0/0	0/0	16/8
11 (7)	Anti-α	16/32	2/4	64/128	0/0	0/0	16/8
12 (8)	Anti-γ1γ2	32/32	0/0[a]	0/4[a]	4/4	0/0	16/4
13 (8)	Anti-γ1γ2	32/32	0/4	0/1	4/4	0/0	8/8
14 (8)	Anti-γ1γ2	32/D[b]	0/D	0/D	4/D	0/D	8/D
15 (9)	Anti-γ1γ2	16/32	0/2	0/0	4/4	0/0	8/16
16 (9)	Anti-γ1γ2	16/D	0/D	4/D	2/D	0/D	8/D

[a] All anti-γ1γ2-treated mice showed atypical, additional faint precipitin bands with both anti-γ1 and anti-γ2 at one or both assays, which differed from IgG1 and IgG2 by position in Ouchterlony gel diffusion.

[b] Died prior to second assay.

[c] Numerical averages of individual highest reciprocal serum dilutions producing distinct precipitin lines against standardized anti-Ig antisera or purified mouse Ig in Ouchterlony gel diffusion.

Fig.II. (16) Effect of Anti-μ Antiserum Dose and Injection Schedule on Immunoglobulin Levels in Mice

Group No.	Phenotype[a] (No. Mice)	Suppressive Treatment			Day of Assay		Mean Ig or Antibody Level[b] Assay 1/Assay 2				
		Serum	Days	Total ml	No. 1	No. 2	IgM	IgG1	IgG2	IgA	Anti-μ
1	BALB/c (3)	NRS	0–15	0.47	24	46	27/32	260/260	64/170	1/8	ND[c]
1a	BALB/c (3)	Anti-μ	0–15	0.47	24	46	0/27	32/150	27/64	0/8	ND
2	BALB/c (4)	NRS	0–48	1.25	42	58	32/32	1020/1540	96/160	10/10	ND
2a	BALB/c (4)	Anti-μ	0–48	1.25	42	58	0/0	96/770	9/8	2/10	ND
3	BALB/c (3)	NRS	0–42	3.15	32	47	32/32	260/1020	48/64	4/8	ND
3a	BALB/c (4)	Anti-μ	0–42	3.15	32	47	0/0	2/24	1/1	0/0	16/8
4	LM (10)	NRS	0–56	4.40	32	57	30/32	640/870	67/140	5/15	ND
4a	LM (6)	Anti-μ	0–56	4.40	32	57	0/0	64/280	6/9	0/5	27/21
5	Nu (10)	NRS	0–56	4.40	32	57	29/32	8/14	27/93	2/4	ND
5a	Nu (6)	Anti-μ	0–56	4.40	32	57	0/0	5/7	4/4	0/0	24/21

[a] LM, littermate; Nu, nude.

[b] Numerical averages of individual highest reciprocal serum dilutions producing distinct precipitin lines against standardized anti-Ig antisera or purified mouse Ig in Ouchterlony gel diffusion.

[c] Not done.

from anti μ induced suppression of Ig levels was examined on day 16 and on day 31 following the termination of treatment.(see Fig.III). After 31 days of coasting (no treatment), no recovery of IgM was seen either in nude mice or littermates despite apparent disappearance of anti μ antibodies in the sera. The IgG levels in the anti μ treated littermates of nude mice were seen to approach levels in control mice while the IgG levels in nude mice showed no recovery. Thus, it was concluded that anti μ suppression in nude mice differed from that in normal mice only quantitatively, i.e. nude mice suppressed more easily and to a greater degree with less ability to recover. The degree to which different Ig levels were suppressed by neonatally initiated anti μ treatment and the persistance of the suppression, was seen to be dependent on the total dose of antiserum used and the suppressive schedule employed.

Fig.III. (16) Recovery of Immunoglobulin Levels During Post-treatment Coasting Period in Mice Treated with Anti-μ Antiserum

Group No.	Phenotype[a] (No. Mice)	Suppressive Treatment			Day of Assay		Mean Ig or Antibody Level[b] Assay 1/Assay 2				
		Serum	Days	Total ml	No. 1	No. 2	IgM	IgG1	IgG2	IgA	Anti-μ
3	BALB/c (3)	NRS	0-42	3.15	77	107	32/32	1020/1020	130/130	8/8	ND[c]
3a	BALB/c (4)	Anti-μ	0-42	3.15	77	107	0/3	72/190	2/50	2/5	0/0
4	LM (7)	NRS	0-56	4.40	72	87	32/32	730/730	140/220	18/25	ND
4a	LM (4)	Anti-μ	0-56	4.40	72	87	0/0	340/540	7/14	7/11	12/0
5	Nu (4)	NRS	0-56	4.40	72	87	32/32	26/28	190/220	4/4	ND
5a	Nu (2)	Anti-μ	0-56	4.40	72	87	0/0	4/8	0/0	0/0	4/0

[a] LM, littermate; Nu, nude.
[b] Numerical averages of individual highest reciprocal serum dilutions producing distinct precipitin lines against standardized anti-Ig antisera or purified mouse Ig in Ouchterlony gel diffusion.
[c] Not done.

C. Anti IgG_1, IgG_2, IgA Suppression in Mice:

Sera directed to the other classes of Ig have not been as powerful in humoral Ig suppression as the anti μ serum. Treatment of nude mice with anti IgG serum produced a distinct suppression of both IgG_1 and IgG_{2a} levels (16,17). There were no detectable effects on IgM or IgA levels with anti IgG treatment (see Fig.IV). Levels of IgG_1 and

Fig.IV. (16) Immunoglobulin Levels in Mice Treated with Anti-α or Anti-γ1γ2 Antiserum During and Following Treatment.

Group No.	Phenotype[a] (No. Mice)	Suppressive Treatment			Day of Assay		Mean Ig or Antibody Level[b] Assay 1/Assay 2				
		Serum	Days	Total ml	No. 1	No. 2	IgM	IgG1	IgG2	IgA	Anti-Ig
4	LM (10)	NRS	0-56	4.40	32	57	30/32	640/870	67/140	5/15	ND[c]
4b	LM (6)	Anti-α	0-56	4.40	32	57	32/32	620/1150	240/340	0/19	8/0
4c	LM (5)	Anti-γ1γ2	0-56	4.40	32	57	26/32	230/260	82/90	4/11	0/0
4	LM (7)	NRS	0-56	4.40	72	87	32/32	730/730	140/220	18/25	ND
4b	LM (6)	Anti-α	0-56	4.40	72	87	32/32	770/850	290/290	15/21	0/0
4c	LM (5)	Anti-γ1γ2	0-56	4.40	72	87	32/32	510/610	150/260	16/22	0/0
5	Nu (10)	NRS	0-56	4.40	32	57	29/32	8/14	27/93	2/4	ND
5b	Nu (5)	Anti-α	0-56	4.40	32	57	26/32	3/5	45/140	0/0	14 8
5c	Nu (5)	Anti-γ1γ2	0-56	4.40	32	57	26/32	0/2	1/2	4/4	10 9
5	Nu (4)	NRS	0-56	4.40	72	87	32/32	26/28	190/220	4/4	ND
5b	Nu (4)	Anti-α	0-56	4.40	72	87	32/32	11/16	220/220	0/3	1 0

[a] LM, littermate; Nu, nude.
[b] Numerical averages of individual highest reciprocal serum dilutions producing distinct precipitin lines against standardized anti-Ig antisera or purified mouse Ig in Ouchterlony gel diffusion.
[c] Not done.

IgG_2 in nude mice showed little ability to recover from anti IgG suppression, whereas IgG_1 and IgG_2 levels in littermates showed substantial recovery even during treatment. Manning (16,17) also examined the ability of IgA levels in nude mice and their littermates to recover from anti IgA induced suppression (see Fig.I, IV). The anti IgA treatment did not affect IgM or IgG levels in the nude mice or their littermates. Nude mice were shown to be more sensitive to anti IgA suppression than their littermates. Levels of IgA in nude mice remained suppressed after cessation

of anti IgA treatment but IgA levels in littermates were shown to recover around 57 days after termination of the treatment.

D. Effects of Anti μ Treatment on Lymphocyte Surface Ig:

Studies with immunofluorescent antisera have further detailed the effects of anti μ on lymphoid tissue. Murgita's (13) studies on 30 day old mice given long term anti μ treatment from birth, revealed the presence of spleens completely devoid of all Ig containing cells. The spleens of 8-9 weeks old mice treated with anti μ from birth contained the same number of follicles as control animals but were completely devoid of germinal centres. When the spleens of control animals were stained with fluorescent anti Ig, they showed large clusters of brightly stained plasma cells. Spleen cells of experimental mice (anti μ treated) were more easily stained by anti IgG_1 than by anti IgM. In general there was a ten-fold reduction in the number of fluorescent stained cells in experimental versus control animals. It was a curious observation that the number of cells staining with anti IgG_2 in experimental animals was 3 times higher than the number of cells staining with anti IgG_1 despite the fact that the animals had higher levels of IgG_1 in the serum.

In the anti μ treated mice the gross appearance of the thymus showed a normal medulla and cortex. The spleen was reduced in size and the intestine appeared normal. However, further analysis of the intestine showed that there was a normal mucosa but the villi had a complete absence of plasma cells.

E. Relationship Between Surface Receptor and Secreted Ig Product:

Anti Ig reagents have been used to examine the relationship between the Ig receptor on the surface of the cell (B) membrane and the class of Ig to be secreted later. It had been formerly believed that the "receptor

equals product" (26), i.e. the class of the surface Ig will be the same class of the Ig secreted by the class. Thus, saturation of a receptor of a given class with a specific antiglobulin should suppress production of Ab of only that Ig class after antigenic stimulation. However, this prediction was found to be strictly correct only for anti IgA which suppressed only the IgA response (26). Pierce et al. (20,27) demonstrated that anti μ could reduce all classes (IgM, IgG_1, IgA) of Ab response to SRBC to less than 10% of control levels.

(a) Suppression of Direct and Indirect PFC.

Several other studies (20,21,28) have shown that in vivo anti μ treatment of newborn mice inhibits the direct and indirect PFC response of mouse spleen cells to SRBC. Treatment of newborn mice with anti IgG serum produced a partial reduction in the direct plaque response but eliminated the indirect plaque response to SRBC. In addition, either anti IgG_1 or anti IgG_2 serum could suppress the production of both classes of Ig, suggesting that the sera contained cross-reacting antibodies or the same cell had more than one type of receptor. The fact that only anti μ could suppress the direct plaque response suggests that the unprimed B cell functional receptor is almost exclusively of the μ class. Thus, virgin precursor cells could have the μ receptor and the γ receptors would appear only after differentiation events provoked by antigen.

(b) Inhibition of Rosette Forming Cells.

The effects of anti μ treatment on rosette forming cells (RFC) have also been examined. A RFC is defined as a cell that binds particulate Ag, usually heterologous red cells, or antigens coupled to red cells. Anti μ and anti γ reagents were used to examine the formation of rosettes in mouse spleen cell suspensions taken at varying intervals after SRBC