MURINE IgE ANTIBODY RESPONSES TO DIVERSE CONTACT SENSITIZING AGENTS

BY

MITCHELL KEITH KENNEDY

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

of

MASTER OF SCIENCE

Department of Immunology
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ABSTRACT

A study of the murine hapten-specific IgE response to diverse contact sensitizing agents was undertaken. Numerous strains with various H-2 haplotypes and non-H-2 (background) genes were "skin painted" with picryl chloride and their TNP-specific IgE responses were assessed. It was determined that strains with H-$2^{a,k,s,q \text{ and } y1}$ haplotypes on non-B10 backgrounds were high IgE responders to picryl chloride. Further, it was seen that of the strains tested only CBA/J (H-2^{k}) mice produced high IgE responses to contact sensitization with NP-O-SUCC and FITC. An exploration of the fine specificity of the NP-specific response revealed that NP-O-SUCC contact sensitized mice could be boosted with NIP-O-SUCC. Therefore, there is suggestive evidence that the repetoire of lymphocytes which mediate the IgE and inflammatory responses to contact sensitization are not the same.

Cell transfer experiments demonstrated that both lymph node and spleen cells from contact sensitized donors could adoptively transfer the TNP-specific IgE response to irradiated naive recipients. This is not the case in the inflammatory reaction of the contact sensitivity response. Hence, more evidence to define distinct lymphocyte populations for the inflammatory and IgE responses to contact sensitization to picryl chloride was generated. Other experiments demonstrated that the TNP-specific IgE response to contact sensitization with picryl chloride could be boosted by plastic-adherent PEC. These results may be interpreted as at least suggestive evidence that a macrophage-like cell is responsible for presenting antigen in the induction of the IgE response to contact sensitization. The i.v. injection of TNP-derivitized spleen cells was determined to suppress the TNP-specific IgE response to picryl chloride in another experiment. Such results may be viewed as evidence that T suppressor cells are inducible in the response. Similarly, the i.v. injection of cyclophosphamide (which selectively abolishs T suppressor cells) enhanced the response. By contrast the i.v. injection of normal spleen cells enhanced the response. The results of the present study and their significance is discussed with a view to determining whether or not the IgE response to contact sensitization would serve as a good model for studying the cellular and genetic regulation of the IgE response in general.

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ABBREVIATIONS

Ab	antibody
ABA	azobenzenearsonate
adh	adherent
Ag	antigen
ALS	anti-lymphocyte serum
$Al(OH)_3$	aluminum hydroxide
APC	Antigen Presenting Cell
ATx	adult thymectomy
cm	centimeter
Cy	cyclophosphamide
d	day
det	determinant
DMSO	dimethyl sulfoxide
DNFB	1-fluoro-2,4-dinitro benzene
DNP	2,4-dinitrophenyl
dth	delayed type hypersensitivity
EDTA	ethylenediamine tetraacetic acid
Fc	Fc portion of immunoglobulin molecule
FCA	Freunds Complete Adjuvant
$Fc\varepsilon$	Fc portion of IgE
$Fc\varepsilon R$	receptor for $Fc\varepsilon$
FCS	fetal calf serum (bovine)

FITC	fluorescein isothiocyanate
FPC	Factor Presenting Cell
g	acceleration of gravity
GAT	copolymer L-glutamic acid:L-alanine:L-tyrosine (60:30:10)
GEF	Glycosylation Enhancement Factor
GIF	Glycosylation Inhibition Factor
gm	gram
HBSS	Hank's balanced salt solution
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid
Ia	immune associated antigens
id	idiotype
Ig	immunoglobulin
IgEBF	IgE Binding factor
IgELR	IgE low-responder
IgENR	IgE non-responder
IgEPF	IgE Potentiation factor
IgESF	IgE Suppression factor
IL-1	interleukin-1
i.p.	intraperitoneal
Ir	immune response
i.v.	intravenous
KDa	kilodalton
kg	kilogram
KLH	Keyhole limpet hemocyanin

l	litre
M	molar
MEM	Eagle's minimum essential medium
mg	milligram
MHC	major histocompatibilty complex
ml	milliliter
mm	millimeter
MW	molecular weight
m-Ig	membrane immunoglobulin
Mo	macrophage
NaCl	sodium chloride
N.b.	Nippostrongylus brasiliensis
ND	not done
NIP-O-SUCC	NIP-O-Succinimide ester
NIP	4-hydroxy-5-iodo-3-nitrophenyl acetyl hapten
NP	4-hydroxy-3-nitrophenyl acetyl hapten
NP-O-SUCC	NP-O-Succinimide ester
NSC	normal spleen cells
nsINH	non-specific inhibition factor
OA	ovalbumin
ox	oxazolone (2-phenyl-4-ethoxymethylene-5-oxazolone)
PBS	phosphate buffered saline
PC	phosphoryl choline
PCA	passive cutaneous anaphylaxis
PCl	picryl chloride
PCLF	picryl chloride factor

PEC	peritoneal exudate cells
PFC	plaque forming cell
pI	isoelectric point
PMN	polymorphonuclear
RT	room temperature
SC	spleen cells
s.c.	subcutaneous
Td	thymus dependent
ThF	T helper cell-derived factor
ThFcs	T helper cell-derived factor (specific for contact sensitivity)
Ti	thymus independent
TNBS	2,4,6-trinitrobenzene sulfonic acid
TNBS-F	TNBS factor
TNP	2,4,6-trinitrophenyl hapten
TNP-SC	TNP-modified spleen cells
TsF	T suppressor cell-derived factor
TsFs	T suppressor cell-derived factors
TsFv	T suppressor cell-derived antigen binding chain
wt	weight
(v/v)	volume by volume (e.g. 1% = 1 ml per 100 ml)
(w/v)	weight by volume (e.g. 1% = 1 gm per 100 ml)
µg	microgram
5HT	serotonin
650R	650 rads
^{0}C	degree Celsius

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INTRODUCTION

I. Delayed Type Hypersensitivity And Contact Sensitivity Responses.

A. The Inflammatory Response In Delayed Type Hypersensitivity and Contact Sensitivity Responses

Delayed type hypersensitivity (dth) is a T cell-mediated immune response which leads to inflammation (localized redness, swelling and pain) 24-48 hours after antigen (Ag) challenge. Contact sensitivity is a form of dth which occurs when an animal comes into repeated contact through skin or mucosal surfaces with a chemical capable of coupling to its proteins. As with all other types of dth, contact sensitization was once characterized by a single peak of inflammation initiated by Ag-specific and H-2 restricted lymphokine secreting T cells (T_{lk}) which appear 3-4 days after antigen sensitization [1]. When these T cells encounter antigen in association with H-2 determinants on Antigen Presenting Cells (APC) [1] (in all probability Langerhan's cells [2-3]), they secrete lymphokines [1] which in turn, attract antigen non-specific circulating monocytes to the challenge site. Finally, the monocytes which have differentiated into activated macrophages [4] ingest the debris and other dead tissue.

Although it was originally thought that dth was effected by a single subpopulation of T cells, as described above, Askenase and Van Loveren [1] discovered that dth is actually a biphasic phenomenon consisting of both early, and more pronounced late phases, which peak 2 and 24-48 hours after antigen challenge, respectively. For example, in the contact sensitivity response to picryl chloride (PCl), the biphasic reaction is mediated in part by two distinct subpopulations of T cells (see Figure 1) [5-6]. The first subpopulation, T_{pclf}, appears in the lymph nodes and spleen 1-2 days after optimal sensitization with picryl chloride [6] and

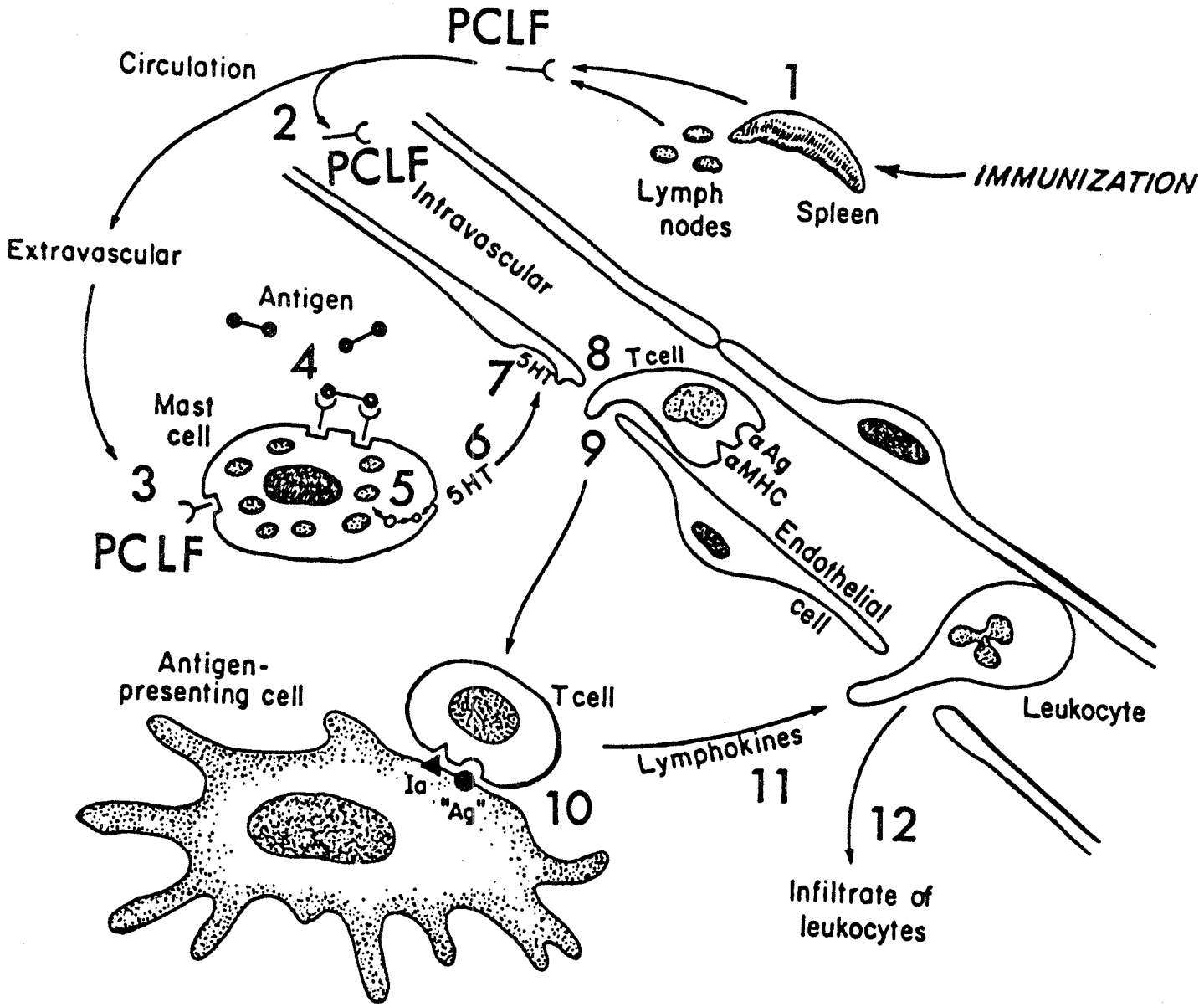

PCLF
Circulation
1
2
PCLF
Intravascular
Lymph nodes
Spleen
IMMUNIZATION
Extravascular
Antigen
4
7
5HT
8
T cell
Mast cell
6
5HT
9
aAg
aMHC
3
5
PCLF
Endothelial cell
Antigen-presenting cell
T cell
Leukocyte
Lymphokines
11
Ia
"Ag"
10
12
Infiltrate of leukocytes

Figure 1

Figure 1: A schematic representation of the steps involved in the elicitation of contact sensitivity responses to "skin painting" with picryl chloride (modified from [1]).

(1) Subsequent to contact sensitization with PCl, T_{pclf} cells (which release PCLF) are generated in the lymph nodes and spleen

(2) PCLF is dispersed throughout the body by the bloodstream

(3) PCLF binds to and thereby sensitizes mast cells

(4) Upon antigen challenge, mast cell bound PCLF is cross-linked by antigen

(5) Mast cells degranulate

(6) Mast cells specifically release serotonin (5HT), but not histamine

(7) Liberated serotonin acts on the serotonin receptors of the endothelial cells of postcapillary venules

(8) Gaps form between endothelial cells

(9) TNP-specific T_{lk} (lymphokine secreting) cells enter the extravascular space

(10) T_{lk} recognize Ia and TNP determinants on APCs (probably Langerhan's cells)

(11) T_{lk} release antigen non-specific lymphokines

(12) The liberated lymphokines attract antigen-specific and antigen non-specific leukocytes.

secretes an antigen-specific T cell factor (PCLF) which binds to tissue mast cells [1]. Cross-linking of bound PCLF with antigen induces the release of serotonin and, subsequently contraction of and gap formation between neighbouring endothelial cells [1]. As a result, circulating leukocytes are then able to enter the extravascular space and mediate the inflammatory reaction as described previously. Currently, it is not known whether T_{pclf} and T_{lk} are distinct mature clones, or merely different stages of a single cell lineage [6]. Collectively, these results suggest that a small molecular weight T cell product, PCLF, circulates through the vascular and extravascular spaces to initiate the dth reaction. Subsequently, very few antigen-specific T cells potentiate this response; hence, the overall mechanism leading to inflammation is extremely efficient.

Ray et al [7] have recently shown that IgE may also make a contribution to the inflammatory response. Thus, they demonstrated that mice, which had been passively sensitized with 2,4-dinitrophenyl (DNP)-specific monoclonal IgE antibodies, developed inflammation upon epicutaneous challenge with 1-fluoro-2,4-dinitrobenzene (DNFB). The IgE-mediated response exhibited the same kinetics as the usual T cell induced reaction and was characterized by mononuclear cell infiltrates. In addition, passively sensitized athymic (nu/nu) mice did not develop a reaction on challenge with DNFB, which indicated a strong T cell dependency. IgE also required an intact Fc portion; presumably to bind to Fc_ε receptors (which are specific for the Fc portion of the IgE molecule) on one or more cell types.

The significance of simultaneous IgE and PCLF-mediated dth is somewhat abstruse. Although, both responses are macroscopically indistingushable, there may be pertinent differences. For instance [1], the cellular infiltrate accompanying the IgE induced response appeared to be richer in eosinophiles. Furthermore the PCLF and IgE-generated responses may have been mediated by distinct types of effector cells, since, for example, experiments using

mast cell deficient mice (W/W^V and Sl/Sl^d) demonstrated that mast cells were required in the former, but not the latter [8-9]. In addition, antigen cross-linking of bound PCLF induced the selective release of serotonin, while antigen cross-linking of bound IgE promoted the release of serotonin and other vasoactive amines [1]. Because of these differences, it is conceivable that the PCLF and IgE-mediated reactions might be complementary systems which insure an inflammatory response in the total or partial absence of the other.

B. The Induction Of Delayed Type Hypersensitivity and Contact Sensitivity Responses

Animals which have been sensitized with a hapten-carrier conjugate produce a secondary dth response to the hapten only upon challenge with the complete sensitizing conjugate [10-11]. This "carrier-specificity" suggests that the induction of dth requires some form of cellular cooperation. Substantial evidence indicates that dth is, in fact, regulated by T helper and T suppressor cells.

Adoptive transfer of dth to soluble protein antigens, certain microbial or viral antigens and some haptens requires I-A identity between donor and recipient [12], while dth responses to other haptens are restricted to K, D and I region identities [12]. Contact sensitizers indiscriminately modify cell surface components including K, I and D antigens thereby inducing $Ly1^+2^-$ T_{CS} (T cells which can adoptively transfer the contact sensitivity response) restricted to I region compatibility and $Ly1^-2^+$ T_{CS} restricted to K and/or D determinants [12].

Animals can be rendered contact sensitive by "skin painting", injection of chemically conjugated cells or intravenous (i.v.) injection of various reactive compounds. Indicative of the complex and sensitive regulatory mechanisms underlying the contact sensitivity and delayed type hypersensitivity responses, is the delicate balance between response and induction of

tolerance. For example, subcutaneous (s.c.) injection of TNP-modified syngeneic spleen cells (TNP-SC) favors a TNP-specific dth response [13], while i.v. injection of the same modified cells usually generates tolerance to TNP [14].

Recently, it has been demonstrated that the induction of T_{dth} requires an Ag-specific T helper cell (Th_{dth}). These helper cells are Thy 1^+ [15], I-A^+ [15], Ly 1^+2^- [15] and radioresistant [16]. It has further been shown that the cells which induce Th_{dth} bear I region determinants [15]. The role of Th_{dth} in the induction of T_{dth} effector cells may be analogous to the function of T helper cells in triggering the B cells of the humoral response. More specifically, pre-T_{dth} may become tolerized upon encounter with antigen unless stimulated by Th_{dth}. The phenomenon of "carrier-specificity" in dth might be explained by the evidence which indicates that in order to cooperate with T_{dth}, both Th_{dth} and T_{dth}must recognize determinants on the same antigen [16].

Ly1^+2^-, I-A^+, I-J^- cells (characteristic of T helper cells) obtained from contact sensitized mice produce a soluble factor in vitro (ThF_{cs}) which can specifically potentiate the contact sensitivity response [17]. It has been proposed that ThF_{cs} may act by binding to haptenated cells via its antigen-binding site and augmenting the immune response by virtue of its I-A determinants (in effect associating antigen with I-A determinants) [17]. Alternatively, ThF_{cs} may bind to APCs and thereby enable the APCs to release non-specific mediators upon contact with antigen in the context of major histocompatibility complex (MHC) determinants [17]. A subpopulation of T cells (T_{prlf}) which proliferate upon reexposure to antigen <u>in vitro</u> can be isolated from dth primed mice [15]. It has been speculated that Ly1^+2^-, I-A^+ T_{prlf} [15] are

identical to Th_{dth}. However, some evidence suggests that T_{prlf} are less sensitive to the effects of antigen-specific Ts cells, than Th_{dth} [18].

C. The Regulation Of Delayed Type Hypersensitivity and Contact Sensitivity Responses

Numerous studies indicate that humoral [19], helper T cell [20-21], cytotoxic T cell [22-23], proliferative T cell [24-25], contact sensitization [19,26] and dth responses [19,26] are regulated by Ag-specific T suppressor (Ts) cells. However, partially because various researchers have examined diverse models of Ts cell induction to numerous antigens, no comprehensive T cell circuit has been proposed which incorporates all available data. Nevertheless, Ts cell circuits do exist in the contact sensitivity responses to NP (perhaps the best understood system to date) and TNP (the system most relevant to this study) and these circuits appear to have some similarities.

The contact sensitivity response to NP and TNP have been extensively studied and found to be regulated by distinct subpopulations of T suppressor cells (see Figures 2 and 3). Each subpopulation of cells (specific for either antigen) can be characterized by (i) binding specificity (Ag or idiotype), (ii) surface phenotype, (iii) genetic restrictions (H-2 or Igh) of the cells or T suppressor cell derived factors (TsF) and (iv) the stage (induction or effector) of the contact sensitivity response the cells modulate. Descriptions of these T cell subpopulations, the factors they secrete, and the roles the cells and their factors play in the regulation of the contact sensitivity response are briefly presented below.

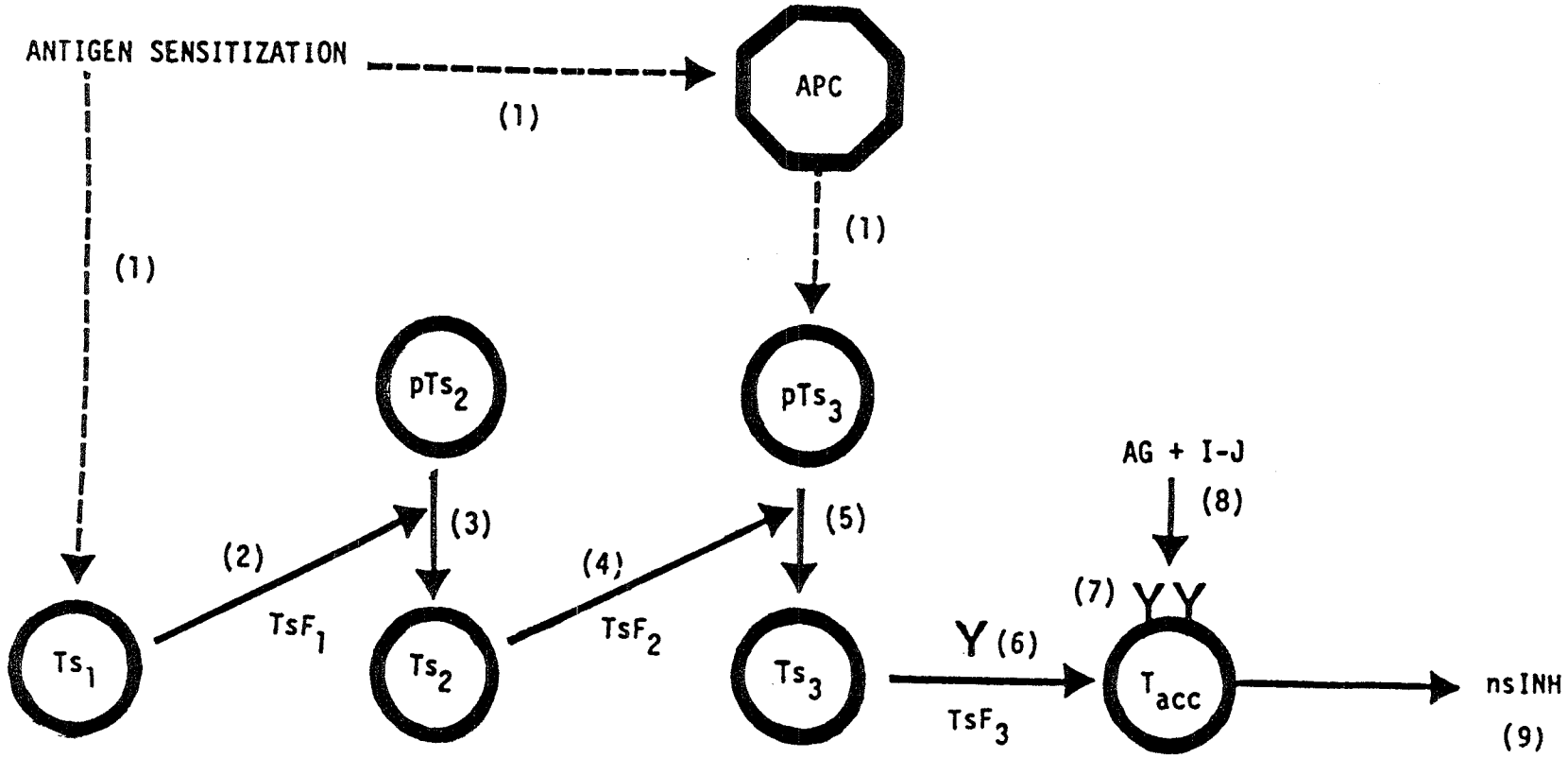

ANTIGEN SENSITIZATION
(1)
APC
(1)
(1)
pTs2
pTs3
AG + I-J
(8)
(7)
(2)
(3)
(4)
(5)
TsF1
TsF2
Ts1
Ts2
Ts3
Y (6)
TsF3
Tacc
nsINH
(9)

Figure 2

Figure 2: Schematic representation of the T suppressor cells which regulate the dth and contact sensitivity responses to NP.

(1) Antigen stimulation induces Ts_1 cells

(2) Ts_1 cells release TsF_1

(3) TsF_1 prompts pre-Ts_2 cells (pTs_2) to mature into Ts_2

(4) Mature Ts_2 cells release TsF_2

(5) TsF_2 prompts pre-Ts_3 (pTs_3) cells to mature into Ts_3 cells

(6) Mature Ts_3 cells release NP-specific TsF_3

(7) TsF_3 binds to antigen non-specific Tacc cells

(8) NP in association with I-J products cross-link Tacc bound TsF_3

(9) Tacc release non-specific inhibition factors (nsINH) which suppress the efferent and afferent limbs of the dth and contact sensitivity response

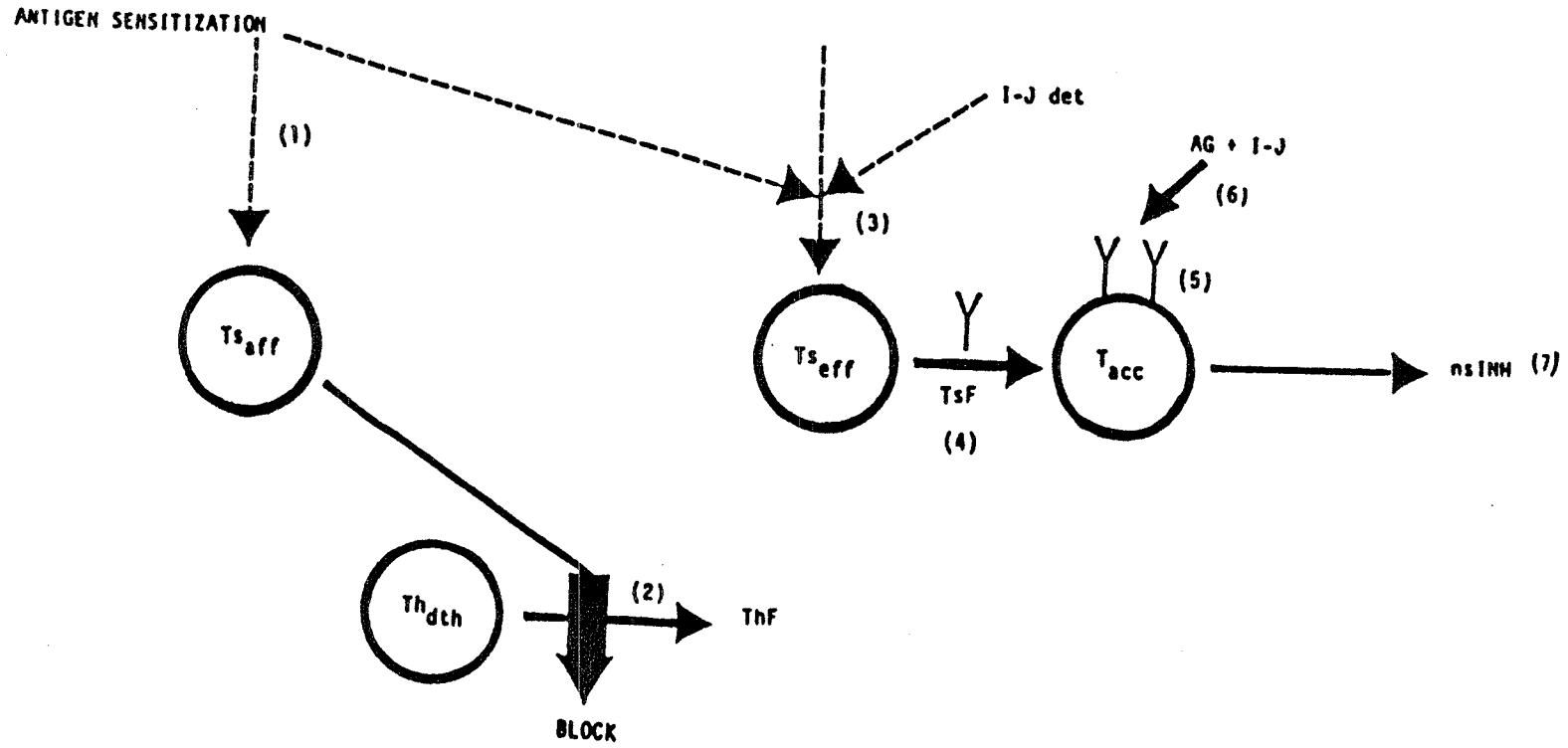

ANTIGEN SENSITIZATION
(1)
I-J det
(3)
AG + I-J
(6)
(5)
Ts_{aff}
Ts_{eff}
TsF
(4)
T_{acc}
nsINH (7)
Th_{dth}
(2)
ThF
BLOCK

Figure 3

Figure 3: Schematic representation of the T suppressor cells involved in the regulation of the dth and contact sensitivity responses to TNP

(1) Antigen sensitization induces Ts_{aff} cells

(2) Ts_{aff} block the afferent limb of the responses by inhibiting the release of ThF by Th_{dth}

(3) Antigen sensitization also induces Ts_{eff}, however unlike the induction of Ts_{aff}, Ts_{eff} induction require Ag in the context of I-J determinants

(4) Ts_{eff} release antigen-specific TsF

(5) TsF binds to antigen non-specific Tacc

(6) Antigen in association with I-J determinants cross-link Tacc-bound TsF

(7) Tacc release non-specific inhibition factors (nsINH) which inhibits both the afferent and efferent limbs of the dth and contact sensitivity responses.

D. The Regulation of Delayed Type Hypersensitivity and Contact Sensitivity Responses To NP

A T cell (Ts_1) which can impede the induction of dth to NP in naive recipients [27] has been isolated from mice injected previously with NP-coupled spleen cells [19,28]. Ts_1 (see Figure 2) are generated by the injection of NP-coupled spleen cells [27], $I\text{-}J^+$ [29], $Ly1^+2^-$ [29], express idiotypes which cross-react with IgV idiotypes [29] and bear antigen binding receptors [27]. Hybridomas of NP or azobenzene arsonate (ABA)-specific Ts_1 cells have also been generated. Furthermore these hybridomas yielded TsF which were $I\text{-}J^+$, $idiotype^+$, Ig^- and which possessed antigen binding sites [30-31].

TsF_1 acts by recruiting a second subpopulation of Ts cells (Ts_2) (see Figure 2) [30-31] possibly by helping pre-Ts_2 cells mature into Ts_2 [32]. While TsF_1 is not H-2-restricted, it appears to be Igh-V-restricted because the Ts_2 cells it induces are Igh-V-restricted [31]. At least one hybridoma-derived TsF_1 was able to suppress both plaque forming cell (PFC) and T cell-mediated contact sensitivity responses through a second subpopulation of Ts (Ts_2) [32].

As described above, NP-specific TsF_1 induces the activation of Ts_2 cells, which in turn can suppress the effector stage of dth, contact sensitivity and PFC responses [30-31]. In addition, Ts_2 are functionally restricted by the I-J genotype of the plastic-adherent, $Thy1^-$ Factor Presenting Cell (FPC) [33] (probably a macrophage) upon which TsF_1 must bind in order to recruit Ts_2. Ts_2 are Igh-restricted [34-35], $Ly2^+$ [34-35], $I\text{-}J^+$ [34-35] and bear anti-idiotype receptors [36-37] which may act as a bridge to interact with Ag-specific, idiotype positive Ts_1 and Ts_3 cells [33]. Similarly, one functionally NP-specific hybridoma has been shown to be antiidiotypic, $I\text{-}J^+$ and to secrete an $I\text{-}J^+$, Igh-restricted suppressor

factor (TsF_2) [38].

Although Ts_3 are induced upon antigen stimulation (see Figure 2), they remain inactive until further stimulated by Ts_2 or TsF_2 [39] in a Igh-restricted manner [40]. In dth responses to NP and ABA, Ts_3 are antigen-specific [39,41], bind antigen [39,41], $Ly2^+$ [41]. Ts_3 suppress the efferent stage of the immune response [42] in an H-2I-restricted manner [39]. The antigen presenting cells (APC) which induce the third subpopulation of Ts (Ts_3) are plastic-adherent [19,43], $Thy1^-$ [19,43], phagocytic [19,43], FcR^+ [19,43], $I\text{-}A^+$ [44] and $I\text{-}J^+$ [45-46].

A soluble suppressor factor (TsF_3) (see Figure 2) has been isolated from a NP-specific Ts_3 hybridoma [42]. In addition, a pre-Ts_3-derived hybridoma has been shown to contain cytoplasmic TsF_3 which is not secreted until the cells are stimulated by TsF_2 [47]. TsF_3 binds to antigen [42], is $I\text{-}J^+$ [42] and restricted to the I-J genotype of the APC which induced Ts_3 [48]. NP-specific TsF_3 [49] {similar to Keyhole limpet hemocyanin (KLH)-specific TsF_3 [50-51]} is composed of 2 chains [32] (i) a 28 KDa, $I\text{-}J^+$ chain and (ii) a 35-45 KDa Ag binding chain. In contact sensitivity TsF_3 act upon accessory T cells (Tacc) (see Figure 2) [19], while B cells serve as the direct or indirect target in the humoral response [32].

E. The Regulation Of Delayed Type Hypersensitivity And Contact Sensitivity Responses To TNP

A subpopulation of Ts cells (Ts_{aff}) which suppresses the induction (or afferent) stage of dth and contact sensitivity to TNP can be isolated from mice "painted" with picryl chloride or injected i.v. with TNP-modified spleen cells [52]. Ts_{aff} seem to be analogous to Ts_1 in the NP-specific system. Thus, both Ts_{aff} bind to antigen (see Figure 3) and both are $Ly1^+2^-$ [53]. In addition, both afferent-acting Ts cells can be distingished from their efferent-acting counterparts by their resistance to adult thymectomy and sensitivity to cyclophosphamide treatment given prior to immunization [54]. However, unlike NP-specific Ts_1, there is some evidence to indicate that TNP-specific Ts_{aff} may not induce an efferent-acting Ts, but rather act directly by blocking the production of Ag-specific T helper factor (ThF).

A subpopulation of Ts cells (Ts_{eff}) which specifically suppress the efferent limb of dth to TNP are similar to NP-specific Ts_3 both in action and function. For example, both cells are $Ly2^+$ [34,55], $I\text{-}J^+$ [34] and bind antigen specifically [42,56]. In addition, both efferent-acting Ts cells are induced by antigen in the context of I-J determinants (Ag +I-J) [48,57] and release an I-J restricted suppressor factor (TsF) (see Figure 3) [58].

Nevertheless, despite the similarities between the NP and TNP-specific systems there is still some confusion concerning the production of TsFs. One T cell-derived suppressor factor appears to be released by a hybridoma derived from a single T cell [32,50], while other TsFs are composed of subunits liberated from two or more T cell subpopulations [59-62]. NP-specific TsFs are composed of at least two types of polypeptide chains; (i) an Ag binding chain (TsFv) and (ii) an $I\text{-}J^+$ chain [32,49].

TNP-specific TsF_{eff} (T suppressor cell-derived factor specific for effector stage of contact sensitization response) appears to consist of at least two serologically distinct

components, TNBS-F and PCLF [63]. TNBS-F has a molecular wieght of 35-75 KDa [60] and is produced by $Ly2^+$, $I\text{-}J^+$ T cells [59] which can be isolated from mice previously injected i.v. with TNBS [59]. The second factor, PCLF, is secreted by $Ly1^+$, $I\text{-}J^-$ T cells [59,64] obtained from mice "skin painted" with picryl chloride. In its oligomeric form PCLF combines with TNBS-F to form TNP-specific TsF [64]. By contrast, in its monomeric form (MW = 70 KDa) PCLF can passively contact sensitize mice to TNP [8-9] (see page 2). Thus, it appears that the multimeric state of PCLF determines its role in the immune response.

The final cell in the TNP-specific suppressor cascade is the Ag non-specific T acceptor cell (Tacc) which requires antigen sensitization (non-specific) [65] for induction. Tacc are $I\text{-}J^+$, $Ly1^-2^+$, Fc^+ and ATx and Cy-sensitive [65-66]. When Ag-specific, TsF molecules are bound to Tacc cells and subsequently cross-linked by antigen in the context of I-J [67], T_{acc} release non-specific inhibitors (nsINH). Evidence indicates that antigen and I-J determinants are recognized separately by Tacc bound TsF [67].

The non-specific inhibition factors (nsINH), which have been demonstrated to suppress the afferent and efferent stages of dth in an antigen non-specific manner, are identical, or at least very similar. Both are $I\text{-}J^+$, derived from T_{acc} cells, have a MW of 50-60 KDa and possess a pI = 6.8 [68]. The targets and mechanisms of nsINH have yet to be determined but should prove to be of considerable interest.

II. The Production And Regulation of IgE

IgE production manifests several unique features. For example, IgE antibodies are normally maintained at very low serum concentrations (μg/ml) in comparison to the serum levels of the other classes of immunoglobulins (e.g. IgG at mg/ml) [69]. Secondly, antigens which normally provoke a vigorous IgE response are usually distinct from conventional antigens [70]. Thus, pollen, fungi, food allergens and parasitic worms all elicit pronounced levels of IgE synthesis while bacteria and viruses, which stimulate effective IgG and IgM responses, do not normally induce significant IgE formation [70]. A third distinctive feature of the IgE response is that it occurs in the mucosa of the gastrointestinal and respiratory tracts where IgE antibodies can be most effective against the stimuli that often induce the IgE responses [70]. Thus, IgE can be considered to be a secretory immunoglobulin.

The IgE response is also strikingly thymus dependent. For example, congenitally athymic (nu/nu) mice must be injected with histocompatible thymocytes in order to develop significant IgE titers [71]. Similarly, hapten-primed B cells require the presence of syngeneic carrier-primed T cells to produce a secondary adoptive IgE response upon challenge with a hapten-carrier conjugate [72].

IgE responder mice produce persistent IgE responses to low doses (μg) of antigen, but only transient IgE responses to high doses of antigen [73]. To elucidate the mechanisms underlying these phenomena, Tamura and Ishizaka [73] immunized different groups of BDF1 mice with several doses of antigen and found that their T suppressor cell and B memory cell activities increased with antigen dose. Thus, the transient nature of the IgE response to the higher doses of antigen appeared to be due, at least in part, to the generation of Ag-specific T suppressor cells.

The IgE response also appears to be more sensitive to T cell regulation than the IgG response. For instance, the ratio of the anti-hapten IgE response generated by carrier-primed spleen cells in irradiated adoptive recipient mice, as compared to the response in the intact

primed donor mice, (IgE irradiated recipient/ IgE unirradiated donor) is much greater than the ratio of the analogous IgG responses (IgG irradiated recipient/ IgG unirradiated donor) [74]. These results suggest a much stricter control of IgE vs IgG synthesis by regulating cells in the normal (unirradiated) recipients than the irradiated recipients.

Two theories have been tendered to explain the divergent sensitivities of the IgE and IgG response to T cell regulation: (i) B cells committed to IgE vs IgG production differ in their sensitivities to T cell regulation; (ii) IgG and IgE responses are governed by distinct subpopulations of class-specific T cells.

B lymphocytes utilize a membrane form of immunoglobulin (m-Ig) to serve as receptor for Ag [75-77]. Often, two or more classes of these m-Ig receptors, which manifest identical Ag-specificity [78-79] and light chain isotype [80], are expressed contemporaneously on the cell surface. For example, the vast majority of mature B cells bear both m-IgM (m-μ) and m-IgD (m-δ) [81-83], but minor populations of B cells also express m-IgG (m-γ) [84], m-IgE (m-ε) [85] or m-IgA (m-α) [86-87]. As discussed below, the expression of a given isotype on a B cell may reflect the precommitment of the cell's clonal progeny to the secretion of a particular isotype of antibody.

Spleen focusing assays can be used to characterize the isotypes produced by the descendants of a single Ag-stimulated B cell. With the aid of this technique, Gearhart et al [88] demonstrated that $\mu^+\delta^+$ B cells are multipotential with regards to immunoglobulin isotype. By contrast, there is some evidence to indicate that some B cells may be precommitted to secrete antibodies of a given class. For example, both CBA/N and (CBA/N X Balb/c)F1 male mice produce phosphorylcholine (PC)-specific (T15 isotype$^+$) IgE antibodies but no PC-specific IgG antibodies upon primary immunization with PC-KLH [89]. Thus, it appears that at least some of the B cells precommitted to IgE production are derived from a subset distinct from those precommitted to produce IgG. The precursors of the PC-specific IgE producing cell were, in

fact, later shown to be $\mu^+\delta^+\varepsilon^+$ B cells [89]. Hence, $\mu^{+/-}\gamma^+$, $\mu^{+/-}\varepsilon^+$ and $\mu^{+/-}\alpha^+$ B cells may be precommitted to secrete IgG, IgE and IgA antibodies, respectively.

Mongini et al [90] examined the influence of T lymphocytes on B cell class-switching. Their results suggested that the temporal order of appearance of various IgG subclasses on the progeny of μ^+ B cells stimulated by TNP-Ficoll in the absence of T cells, corresponded directly to the 5' to 3' IgC_H gene order. By contrast, μ^+ B cells, in the presence of T cells, appeared to be able to differentiate directly, and by independent pathways, into $\mu^{+/-}\gamma\delta^+$, $\mu^{+/-}\varepsilon^+$ and $\mu^{+/-}\alpha^+$ B cells. Collectively these results suggest IgE production may be regulated by three distinct and sequential mechanisms, which control (i) class switching of $\mu^{+/-}\delta^+$ and/or $\mu^{+/-}\gamma^+$ B cells into $\mu^{+/-}\varepsilon^+$ B cells, (ii) proliferation of $\mu^{+/-}\varepsilon^+$ cells, and (iii) differentiation of mature $\mu^{+/-}\varepsilon^+$ B cells into IgE-secreting plasma cells.

Some strains of mice, including the SJL and AKR strains, are noteworthy because they typically produce remarkably weak IgE responses concomitantly with normal antibody responses of the other isotypes [91]. However, even these "so-called" IgE non-responder strains (IgENR) demonstrate high and persistent IgE antibody responses following low dose irradiation; treatment with Cy , or injection of anti-lymphocyte serum (ALS) [92]. Moreover, the enhanced IgE antibody reponses are selectively abolished by the injection of histocompatible normal spleen cells (NSC) [92] or thymocytes. Hence, it appears that AKR and SJL mice are poor IgE responders by virtue of a putative and innate class-specific suppressor T cell subpopulation and not because of a genetic incapacity to produce IgE.

Recently, several investigators have demonstrated that there are, in fact, specific subpopulations of T cells (T_ε) which can regulate the IgE response in a class-specific and antigen non-specific fashion. These T_ε cells express receptors for the Fc portion of Ig (FcR) and secrete two forms of IgE binding factors (IgEBF); those which potentiate IgE production

(IgE Potentiation Factor; IgEPF) [93] and those which suppress IgE production (IgE Suppression Factor; IgESF) [94]. T cells, which are isolated from rats 8 days after infection with the helminth Nippostrongylus brasiliensis (Nb) (T cells with low levels of FcR specific for IgE ($Fc_\epsilon R$)) and subsequently stimulated with IgE, produce IgESF, while T cells isolated 14 days after Nb infection (T cells bearing higher levels of $Fc_\epsilon R$) and then stimulated with IgE secrete IgEPF [93]. T cells from immunized rats also secrete IgE-regulating factors upon reexposure to antigen in vitro [95]. The choice of adjuvant used in immunization, however, has a striking effect on the relative amounts of IgESF and IgEPF produced. In view of the fact that the adjuvant $Al(OH)_3$ generally enhances the IgE response in vivo, whereas Freunds Complete Adjuvant (FCA) markedly suppresses the IgE response, it is of interest that T cells isolated from rats immunized with KLH in $Al(OH)_3$ produce predominantly IgEPF upon reexposure to KLH, while T cells isolated from rats sensitized with KLH in FCA secrete primarily IgESF [95-96]. The preceeding observations indicate that IgEBFs may play a significant role in the class-specific regulation of IgE.

Both IgEPF and IgESF are IgEBFs and there is evidence to indicate that IgEPF is simply a more extensively glycosylated form of IgESF [97]. Studies have shown that the glycosylation of IgESF is regulated by two additional factors; one which enhances the glycosylation of IgEBF [96] (Glycosylation Enhancement Factor; GEF) and the other which inhibits this glycosylation [96] (Glycosylation Inhibition Factor; GIF). Hence, the balance between IgEPF and IgESF formation is dependent upon the relative levels of GEF and GIF.

Recent studies have suggested that class-specific and antigen-specific T suppressor cells may act synergistically to regulate the IgE response. Thus, ovalbumin (OA) can stimulate OA-specific $Ly1^+$ T helper cells from OA suppressed mice, to release a factor (Inducer Factor; IF) which, in turn induces the release of IgEBF. OA also provokes OA-specific $Ly2^+$, $I\text{-}J^+$

T suppressor cells to secrete GIF [98]. In combination, these two factors stimulate unprimed $Ly1^+$ T cells to release IgESF [98]. By contrast, OA can stimulate OA-primed T helper cells ($Ly1^+$), from OA primed mice, to secrete IgEBF and GEF which, together, induce unprimed T cells ($Ly1^+$) to form IgEPF [99].

III. Ir Gene Control Of The Immune Response

A. General Considerations

Ir genes are the genetic elements which control the ability of animals to respond to certain antigens. All immune responses to T dependent (T_d) antigens (e.g. humoral responses to T_d antigens, dth, helper T cell, proliferative T cell, Ag-specific cytotoxic T cell and contact sensitivity responses) are under Ir gene control, while responses to T independent (T_i) antigens (e.g. humoral responses to T_i Ags, are not [100-102]. Ir gene products (Ia Ags) play an essential role in the presentation of antigen to unprimed T cells by macrophages [103-105] and the induction of B cells by inducer T cells (see Figure 4) [103-104]. Evidence that the immune response is under genetic control came from the observation that the ability of outbred guinea pigs to produce dth or antibody responses to simple peptides could be mapped to a single genetic locus [106-107]. In the mouse the Ir genes have been mapped to the I-A and I-E subregions of the H-2 complex [100] (see Figure 5).

Ia Ags are composed of 2 polypeptide chains (α and β) encoded by either Eα and Eβ or Aα and Aβ genes (see Figure 5) [103-104]. The complete Ia molecules are found embedded in the plasma membrane of macrophages and B cells (see Figure 5) and seem to function by presenting antigen in context with self-determinants [103-104]. It has yet to be resolved, however, whether T cells recognize antigen and Ia determinants by two distinct receptors (Dual Receptor Model) or by a single receptor (Altered-Self Model) specific for a combination of antigen and Ia determinants (see Figure 6).

Three models have been proposed to explain how Ir genes control the immune response: (i) the Determinant Selection Model, (ii) the Adaptive Differentiation Model and (iii) the Induction of Ts Model. Each of these models and some of the evidence which supports it are briefly examined below.

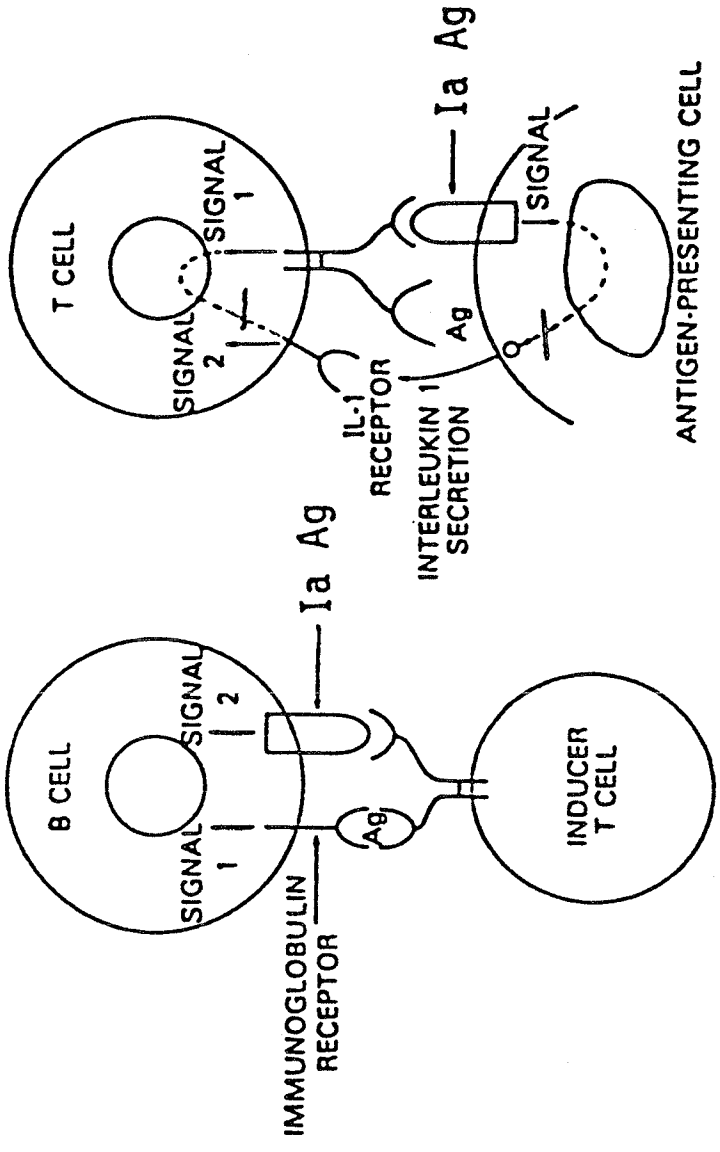


Figure 4: Models for the role of Ia Antigens in B cell And T cell Activation.

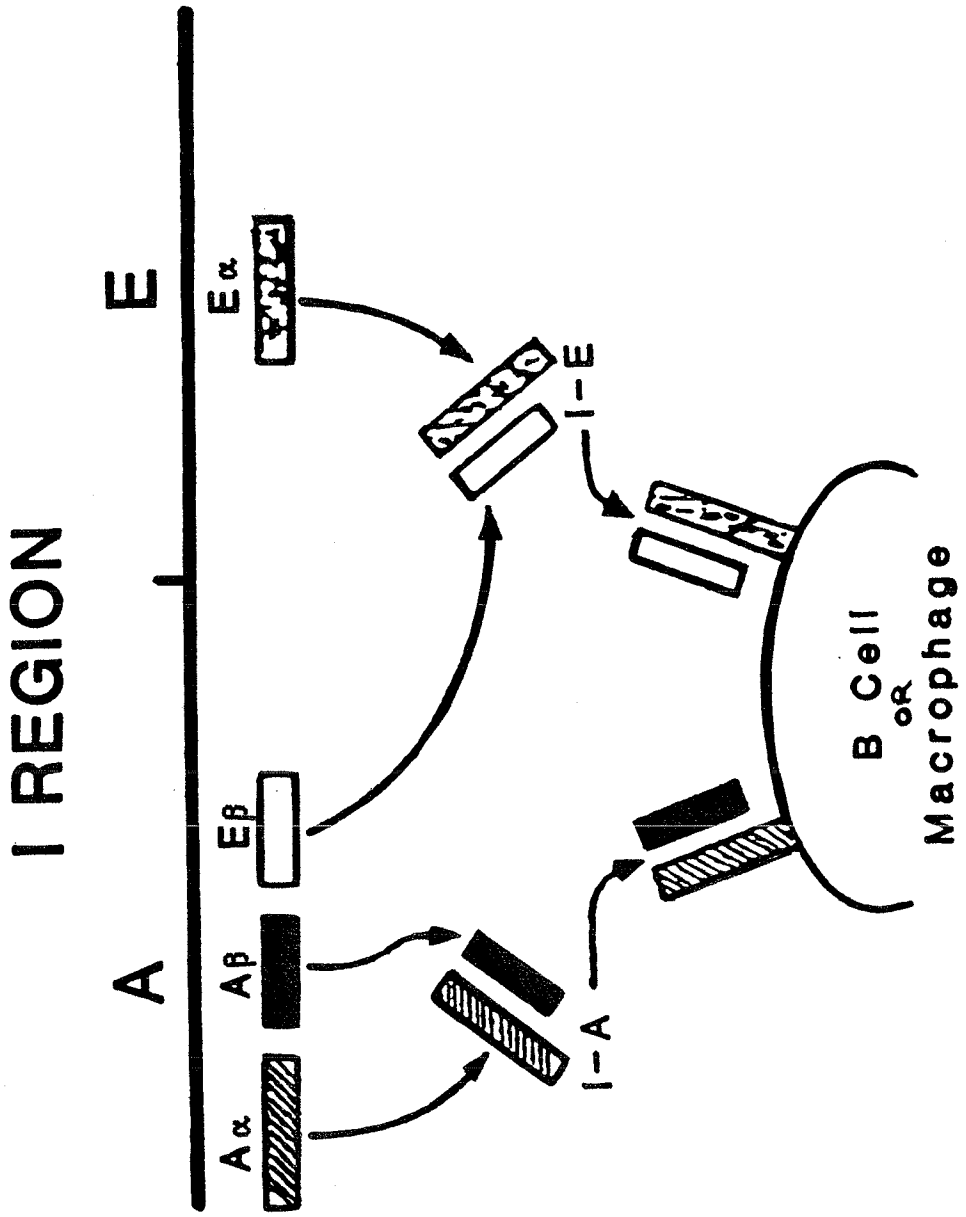
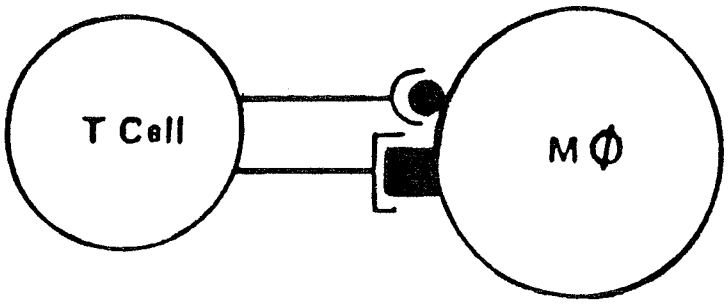


Figure 5: Model Of The Genetic Organization And Expression Of I-A And I-E Antigens On B Cells And Macrophages.

Dual Receptor Model



Altered Self Model

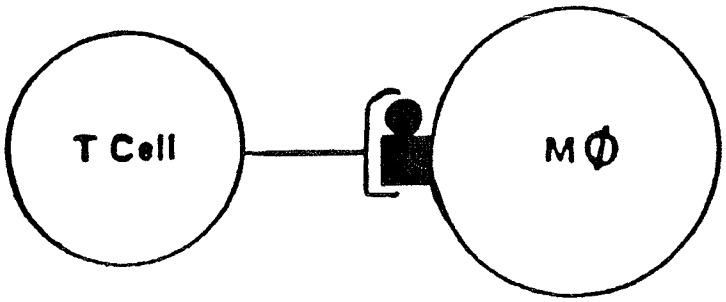


Figure 6

Figure 6: "Dual Receptor Model" and "Altered-Self Model" of T cell recognition.

LEGEND

MØ----------macrophage


----------antigenic determinant


----------Ia determinant

B. The Determinant Selection Model

Originally proposed by Benacerraf [108] the determinant selection model of Ir gene control alleges that immune non-responsiveness occurs because macrophages of the non-responder strain are unable to present the determinants of the antigen in question in an immunogenic form to T cells. Evidence in support of this model stems from studies of the ability of guinea pigs to respond to pork insulin [109-110]. The insulin molecule is composed of two chains (α and β). It was shown that Strain A guinea pigs were able to mount an immune response to the α chain of the insulin while being unresponsive to the β chain. By contrast, Strain B guinea pigs responded to the β chain and were tolerant to the α chain. Thus, the preceding observations indicate that animals with different Ir genes recognize distinct antigen determinants on the same molecule.

C. The Adaptive Differentiation Model

The adaptive differentiation model proposes that the defect in non-responder mice lies with the inability of T cells to respond to certain Ia Ag plus foreign Ag combinations. One version of this model assumes that as stem cells differentiate in the thymus emerging thymocytes with a high affinity for either self Ia or [autologous Ag + self Ia Ag] are depleted, which creates a lacuna in the T cell repertoire. Hence, if these T cells, include a population which would normally respond to a given foreign antigen in association with self Ia Ag, the animal is left unresponsive to that particular antigen.

Corroborative evidence for the adaptive differentiation model stems from experiments using irradiation bone marrow chimeric mice [111]. These animals are created by injecting T cell depleted bone marrow cells from mice of one strain into mice of a second strain, which have been depleted of their own stem cells by prior exposure to irradiation. Subsequently, some of

the bone marrow cells mature into thymocytes in the host, thereby recognizing their own and the host's Ia antigens as self. Parent strain A mice which have been injected with F1 (strain A X strain B) bone marrow cells have been shown to produce a response only to antigens in association with strain A, but not strain B, Ia antigens. Hence, it could be concluded that T cells respond to foreign antigens in association with the Ia antigens found in the enviroment in which they mature.

D. The Activation of T Suppressor Cells Model

This model proposes that some animals do not produce a detectable immune response, not because of an incapacity to respond, but rather because of an induction of antigen-specific T suppressor cells. In other words, some antigens stimulate suppressor mechanisms which overwhelm the responsive mechanism. For example, H-2^s and H-2^q congenic mouse strains are unable to mount a detectable humoral response to a random copolymer of glutamic acid, alanine and tyrosine (GAT) [112-113]. However if either strain is subjected to immune manipulations which quell T suppressor cell mechanisms, a significant anti-GAT response may be seen [114].

IV. Introduction to the Present Study[1]

Contact sensitizing agents can ellicit boosterable IgE responses in the absence of adjuvants. The main objective of the present experiments was to characterize some of these contact sensitization induced IgE responses with a view to evaluating whether or not they could serve as a useful model for studying the cellular and genetic regulation of IgE responses in general. Therefore a brief examination of the induction, regulation and genetics of the IgE response to contact sensitizing agents was carried out.

An initial attempt was made at identifying the cell(s) responsible for the induction of the response. This was accomplished by determining the ability of various haptenated cells to boost the TNP-specific IgE response in sensitized recipients. In addition, in order to diagnose which cells could adoptively transfer the TNP-specific IgE response, spleen or lymph node cells from sensitized donors were injected into naive recipients. As well, to resolve the possible role of suppressor cells in the response to picryl chloride, mice were injected i.v. with TNP-SC or cyclophosphamide.

The ability of a variety of mouse strains, with diverse H-2 and non-H-2 "background" genes, to develop contact sensitization induced IgE responses to picryl chloride, FITC and NP-O-SUCC was also examined in order to investigate the contribution of H-2 and non-H-2 genes to the induction and/or regulation of these IgE responses.

An analysis of antigen fine specificity can provide some indication as to whether or not two types of immune responses are mediated by the same repetoire of cells. Consequently, the fine specificity of the IgE response to contact sensitization with NP-O-SUCC was also investigated.

(1) It should be noted that the present study comprised the second research project undertaken by this student. The first research project involved the examination of the GAT-specific IgE response. However, due to the publication of a paper related to the first project by other authors it was decided to switch to the present study in order to maintain an "originality of research" aspect to the student's M.Sc. programme. Hence, the results described in this thesis constitute about only two thirds of the student's work.

MATERIALS

I. Animals

A/J, AKR, A.SW, CBA, C57BL/6, DBA/1, DBA/2 and B6D2F1 mice were purchased from the Jackson Laboratories, Bar Harbor, Maine, and from the University of Manitoba's breeding vivarium, Gunton, Manitoba. AQR, B10.A(4R) and C3H.OH mice were acquired from the small-animal breeding unit of the University of Alberta, Edmonton, Alberta. C57BL/10, B10.A, B10.A(3R), B10.BR and B10.S mice were obtained from our private colony originally established from breeding pairs generously provided by Drs. F. Bach and C. David. Random bred hooded rats were obtained from the University of Manitoba's breeding vivarium, Gunton, Manitoba. All experimental groups of mice consisted of four sex and age-matched animals.

II. Chemicals and Reagents

2,4,6-trinitrochlorobenzene (picryl chloride; PCl) was purchased from British Drug House Ltd., Poole, England; trinitrobenzene sulfonic acid, in the form of its sodium salt, (TNBS) was obtained from ICN Pharmaceuticals Inc., Cleveland, Ohio; 4-hydroxy-3-nitrophenylacetyl-O-succinimide (NP-O-SUCC) and 4-hydroxy-3-iodo-5-nitrophenylacetyl-O-succinimide (NIP-O-SUCC) were acquired from Biosearch Inc., San Rafael, California; cyclophosphamide and fluorescein isothiocyanate (FITC) were obtained from Sigma Chemical Company, St. Louis, Missouri; sodium borate, sodium azide, boric acid, ethylene diamine tetraacetic acid (EDTA) and ammonium chloride (NH_4Cl) were bought from Baker Chemical Co., Phillipsburg, N.J.; tris-(hydroxymethyl) aminomethane (TRIS), dimethylsulfoxide (DMSO) and sodium carbonate were acquired from Fisher Scientific Co., Fairlawn, N.J., thioglycollate was obtained from Difco Laboratories, Detroit, Michigan; and heparin was purchased from Allen and Hanburys, Toronto, Ontario. Hank's balanced salt solution (HBSS), Eagle's minimum essential medium (MEM) and fetal calf serum (FCS) were obtained from Grand Island Biological Co., Grand Island, New York; HBSS was supplemented with sodium carbonate (4 mM) and MEM was supplemented with HEPES buffer purchased from Calbiochem, LaJolla, California. Sodium metrazoate was obtained from Accurate Chemical and Scientific Corp, Westbury, New York. Ficoll was supplied by Pharmacia, Uppsala, Sweden.

METHODS

A. "Skin Painting" With Picryl Chloride

Both the thorax and abdomen of mice were cleanly shaved with electric animal clippers prior to each application of the contact sensitizing agent. With the aid of a Pipetman 0.1 ml of a 1%, 5% or 10% PCl in absolute ethanol was "painted" onto a fixed area (approx. 2 cm X 2 cm) of shaven skin.

B. Contact Sensitization With FITC, NIP-O-SUCC and NP-O-SUCC

FITC, NIP-O-SUCC or NP-O-SUCC were dissolved in DMSO at a concentration of 70 mg/ml. A total of 0.1 ml of this solution was then injected into 9 sites spaced over approximately 2 cm^2 of abdomen which had been shaved with electric animal clippers. Immediately thereafter a total of 0.1 ml of borate buffered saline (0.02 M borate, 0.15 N sodium chloride, pH = 8.6) was injected subcutaneously into the same 9 sites (located by means of the small swellings caused by the s.c. injection of the antigen).

C. Measurement of Levels of IgE Antibodies

IgE antibodies were measured by means of passive cutaneous anaphylaxis (PCA) in rat skin. At the time of writing evidence had shown that this procedure detected only murine IgE and not murine reaginic IgG antibodies. However, more recently, data obtained from the use of monoclonal antibodies has shown that IgG_1 may make some contribution to the PCA response [115].

Experimental groups consisted of four mice which were matched for sex and age. Test sera were produced by pooling blood, taken in approximately equal volumes from all of the members of the group. Sera were either tested immediatly or frozen as soon as collected and then thawed just prior to testing. Two-fold serial dilutions of test sera were injected intercutaneously into the shaved backs of random bred hooded rats. Approximately 24 hours later the PCA reaction was ellicited by the i.v. injection of 1 ml/mg solution of an appropriate "challenge antigen" (TNP_{15}-BγG, TNP_{15}-BSA, $FITC_{7}$-GγG or NP_{15}-BSA) in phosphate buffered saline (PBS) containing 1% Evans blue. Except where otherwise stated , the PCA titers of all serum samples were evaluated according to the following rubrics: (i) up to 6 samples were titrated on each rat; (ii) when numbers permitted, all serum samples from a particular bleeding of a given experiment were titrated on the same rat; (iii) samples from a particular bleeding were titrated concurrently and in duplicate on two different rats; (iv) most titrations were not repeated, i.e., the values obtained were from a single serial dilution; (v) PCA values for each rat were obtained by the use of a semi-logarithmic graph {abcissa ($\log_{10}$ scale) = serum dilution; ordinate (arithmetic scale) = average diameter of spot}. Values at both the highest serum dilution to yield a spot with a diameter greater than 5 mm and the next highest serum dilution to yield a spot with an average diameter of either less than 5 mm or no spot at all were plotted and a straight line drawn between these two points. The PCA titer was considered to be the value of the abcissa at the point where the plotted line intersected the ordinate at 5 mm. (vi) PCA titers are reported as arithmetic means. (vii) In most cases, PCA titers of a given sample exhibited less than or about two-fold variation. Nevertheless, in the present study, two-fold differences in PCA titers of distinct samples were considered significant when these differences were shown to be reproducible in two or more experiments.

D. Preparation of Peritoneal Exudate Cells (PEC)

Mice were injected intraperitoneally (i.p.) with 3 ml of thioglycollate solution (prepared as described below). Three days later the mice were killed by cervical dislocation and subsequently injected i.p. with 5 ml of HBSS-heparin (1% v/v). The swollen abdomens were gently massaged and the abdominal skin was pulled away, leaving the exposed muscle and peritoneum intact. A small slit was made in the peritoneum and the cellular infusion removed with a Pasteur pipette. The cavity was then washed with ice-cold HBSS-heparin. All cells were pooled, washed 3 times in cold HBSS (no heparin), and counted. These cells were considered to be "whole PEC".

E. Preparation of Adherent PEC

Five X 10^7 whole PEC (prepared as described above) in 5 ml of HBSS were placed into petri dishes. The plates were then incubated at 37^0C for 1 hour, the supernatant was carefully poured off, the plates were then washed 3 times with HBSS at 37^0C and finally reincubated for another hour as before with fresh HBSS. After the second incubation the plates were washed 3 times with HBSS at 37^0C and reincubated at 37^0C for 15 minutes with 5 mls of 6 X 10^{-5} M EDTA. The cells were collected by vigorous pipetting of the EDTA solution and with the aid of a Rubber policeman. Finally, the cells were washed 3 times in ice-cold HBSS, counted and made up to working concentrations.

F. Preparation of Non-Adherent PEC

Non-adherent PEC were collected in the supernatant obtained after the first 1 hour incubation of whole PEC in HBSS. This supernatant was reincubated for 1 hour at 37^0C in fresh

plates and those cells which did not adhere to the second set of plates were collected. These cells were washed 3 times in cold HBSS, counted and made up to working concentrations.

G . Haptenation of Cells with TNP

Cells were haptenated by a method modified from Greene et al [13]. More specifically, equal volumes of cell suspension (5×10^7 cells/ml HBSS) and 10 mM TNBS in HBSS (pH = 7.2) were combined and gently stirred at RT for 30 minutes. The cells were then washed 3 times in large volumes of ice-cold HBSS, counted, and made up to working concentrations.

H. Purification of Spleen Cells (Ammonium Chloride Method)

Mice were killed by cervical dislocation and their spleens were removed and placed into ice-cold HBSS. The organs were disrupted in a loose-fitting teflon homogenizer and the resulting suspension was passed through a wire mesh. The cells were washed 3 times in cold HBSS and then exposed to 0.83% (w/v) NH_4Cl in TRIS-HCl buffer (pH = 7.2) for 4 minutes at room temperature (RT). Finally, the cells were washed 3 times in cold HBSS, counted and made up to working concentrations.

I. Irradiation of Mice

Mice were encased in a plexiglas box and exposed to a Co^{60} source at prescribed distances for the prescribed time.

J. Preparation of Thioglycollate Solution Used to Induce PEC

Thioglycollate medium was dissolved to a concentration of 29.8 gm/l in water, and this solution was boiled for 5 minutes. Subsequently, the thioglycollate solution was autoclaved and then aged in the dark at RT for at least a month prior to use.

K. Purification of Lymphocytes by Ficoll

Mice were killed by cervical dislocation and their spleens and/or lymph nodes removed and placed into ice-cold HBSS. The organs were disrupted by the use of a loose-fitting teflon homogenizer and the resulting cell suspension passed through a wire mesh. The cells were resuspended in HBSS (RT) (the cells of approximately two spleens per 5 ml medium). Five ml of this cell suspension were very carefully layered onto 4 ml of Ficoll solution (10% Ficoll, 9.65% sodium metrazoate, 0.1% sodium azide at RT) in a 15 ml polycarbonate tube. Subsequently, the tubes were centrifuged at 1235 g for 20 minutes at RT. Finally, the cells at the HBSS-Ficoll interface were collected, washed three times in cold HBSS, counted and, then, made up to the required working concentrations.

RESULTS

A. Titers of TNP-specific IgE in CBA/J Mice To Various Doses of Picryl Chloride

In order to determine the antigen dose which elicits the optimal TNP-specific IgE response to picryl chloride, mice were "painted" on days 0, 7 and 37 with various concentrations (0%, 1%, 5% and 10% w/v) of picryl chloride dissolved in absolute ethanol. Mice were bled daily after the first "painting" and weekly after the subsequent "paintings" and then TNP-specific PCA titers of the resulting serum samples were established (as per Methods). As can be seen in Figure 7 none of the groups generated a response after only one "painting" and the negative control (0%) groups (not shown) did not produce significant titers even after a third "painting". In addition, all groups which received picryl chloride showed a measurable TNP-specific PCA response after two "paintings" which could be markedly boosted by a third "painting". In view of the fact that the magnitude of these responses did not manifest a striking dependence on the dose of PCl used to paint the animals, a 5% solution of PCl was used in all subsequent experiments.

B. Anti-TNP IgE Synthesis by Various Mouse Strains in Response to Contact Sensitization with Picryl Chloride

The induction and regulation of dth and contact sensitivity responses involves the presentation of antigen in the context of various self-determinants which are encoded by various genetic loci. It was of interest to establish whether or not the H-2 and non-H-2 (background) genes could influence anti-TNP PCA titers provoked by "skin painting" with PCl. Consequently, various strains of mice were "painted" with a 5% solution of picryl chloride on days 0, 7 and

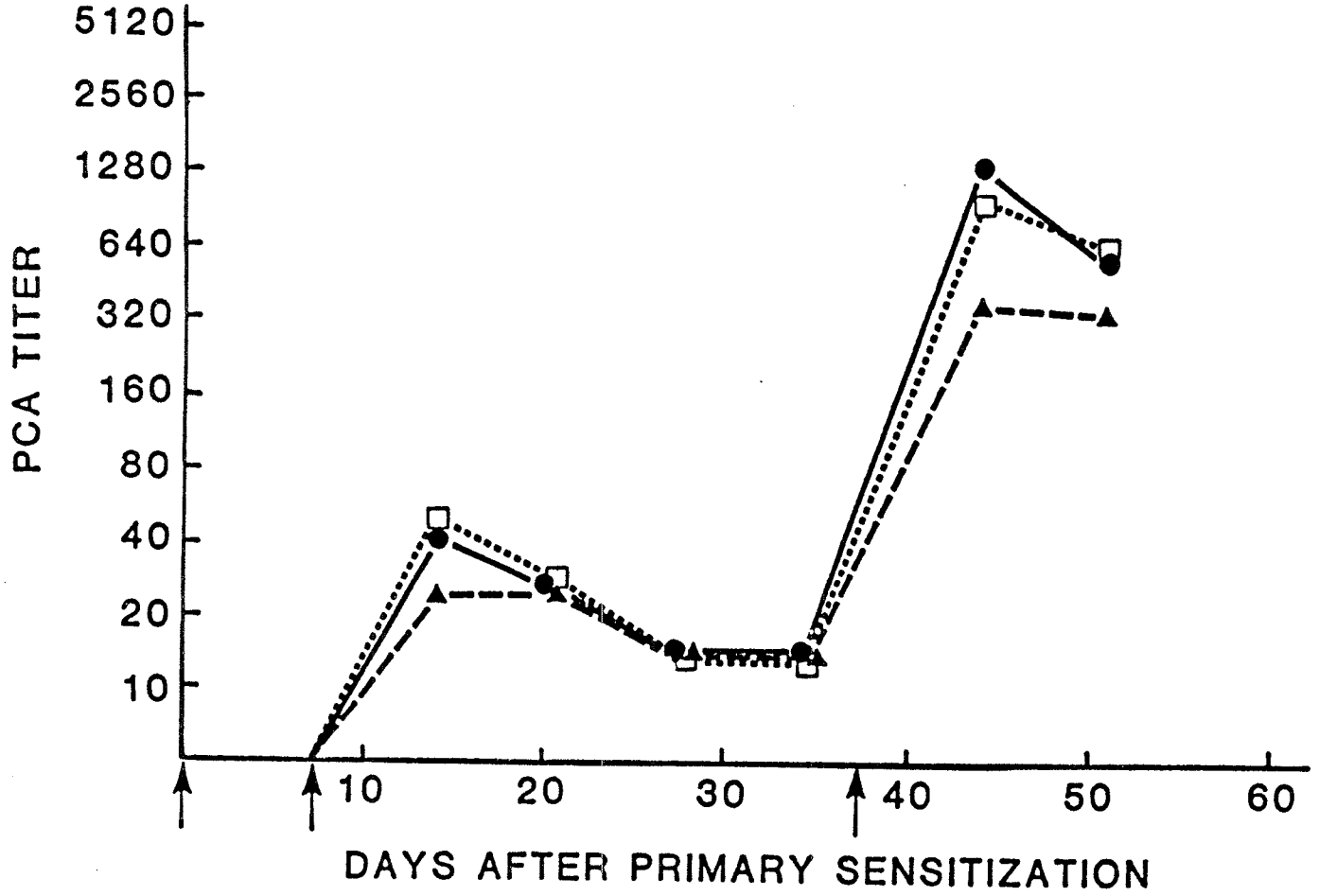

5120
2560
1280
640
320
160
80
40
20
10
PCA TITER
10
20
30
40
50
60
DAYS AFTER PRIMARY SENSITIZATION

Figure 7

Figure 7: Groups of CBA mice were sensitized on days 0, 7 and 37 with 0.1 ml of 1% (▲); 5%(●) or 10% (□) solutions of PCl in ethanol, as described under METHODS. The mice were bled at weekly intervals and the TNP-specific PCA titers of the serum samples were determined.

37, bled days 14, 44 and 51 and the anti-TNP PCA titers of the resulting serum samples were ascertained. Most of the values shown in Table 1 are an average of the PCA titers of serum samples obtained from two groups of mice sensitized in two separate experiments. As can be seen, mice with H-$2^{a,k,s,q \text{ and } y1}$ haplotypes on a non-B10 background produced a measurable response after two "paintings", wheras mice with the H-2^{d} haplotype on a non-B10 background did not. As outlined previously [91], AKR mice are IgE low-responders (IgELR) and this may account for their lack of detectable anti-TNP PCA titers after two "paintings". Genes outside the H-2 complex also play a role in IgE production to PCl "painting" as evidenced by the fact that strains with an H-2^{k} haplotype on a B10 background did not produce significant anti-TNP titers even after two "paintings". In addition, mice with H-2^{b} haplotypes on a non-B10 background (see Figure 8) did not produce a measurable anti-TNP IgE response following the antigen challenge. All of the tested strains, however, displayed a substantial anti-TNP PCA response after a third "painting". Overall, however, even after the third "painting", strains with a B10 background displayed low titers compared to the other strains tested. Furthermore, even within the strains sharing the B10 background it was seen that mice possessing H-$2^{k \text{ and } s}$ haplotypes produced higher TNP-specific PCA titers than those with H-$2^{k/b, k/d \text{ and } b/d}$ haplotypes. Therefore, it appears that that both the MHC and non-MHC background genes play a role in the induction and or regulation of TNP-specific IgE production in picryl chloride "painted "mice.

C. Transfer of TNP-specific IgE Response To Irradiated Recipients

Analyses of the complex networks which regulate the immune response are often facilitated by the transfer of select populations of immunocompetent cells into syngeneic mice whose own immune system has been temporarily inactivated by γ-irradiation. Hence, an

TABLE 1

TNP-specific IgE Synthesis by Various Mouse Strains in Response to Contact Sensitization with PCl(a)

Strain	H-2 HAPLOTYPE							TNP-specific PCA Titer on Day		
	K	Aβ	Aα	Eβ	Eα	S	D	14	44	51
CBA	k	k	k	k	k	k	k	45	430	145
C3H	k	k	k	k	k	k	k	95	ND	ND
A/J	k	k	k	k	k	d	d	40	1600	650
AQR	q	k	k	k	k	d	d	55	955	560
AKR	k	k	k	k	k	k	k	<4	115	5
B10.BR	k	k	k	k	k	k	k	<4	150	40
B10.A	k	k	k	k	k	d	d	<4	80	10
B10.A (3R)	b	b	b	b	k	d	d	<4	90	35
B10.A (4R)	k	k	k	k	b	b	b	<4	80	40
B10.S	s	s	s	s	s	s	s	<2	410	215
DBA/2	d	d	d	d	d	d	d	<2	60	45
A.SW	s	s	s	s	s	s	s	15	660	490
DBA.1	q	q	q	q	q	q	q	10	250	150

(a) Mice were "painted" on days 0, 7 and 37 with the sensitizing dose of PCl and were bled on days 14, 44 and 51. The TNP-specific PCA titers were determined as outlined in METHODS. PCA titers shown are an average of titers obtained in at least two independent experiments. For practical reasons all of the mice listed in this Table were not tested in the same experiment. In most experiments, however, at least one high responder strain served as an internal control.

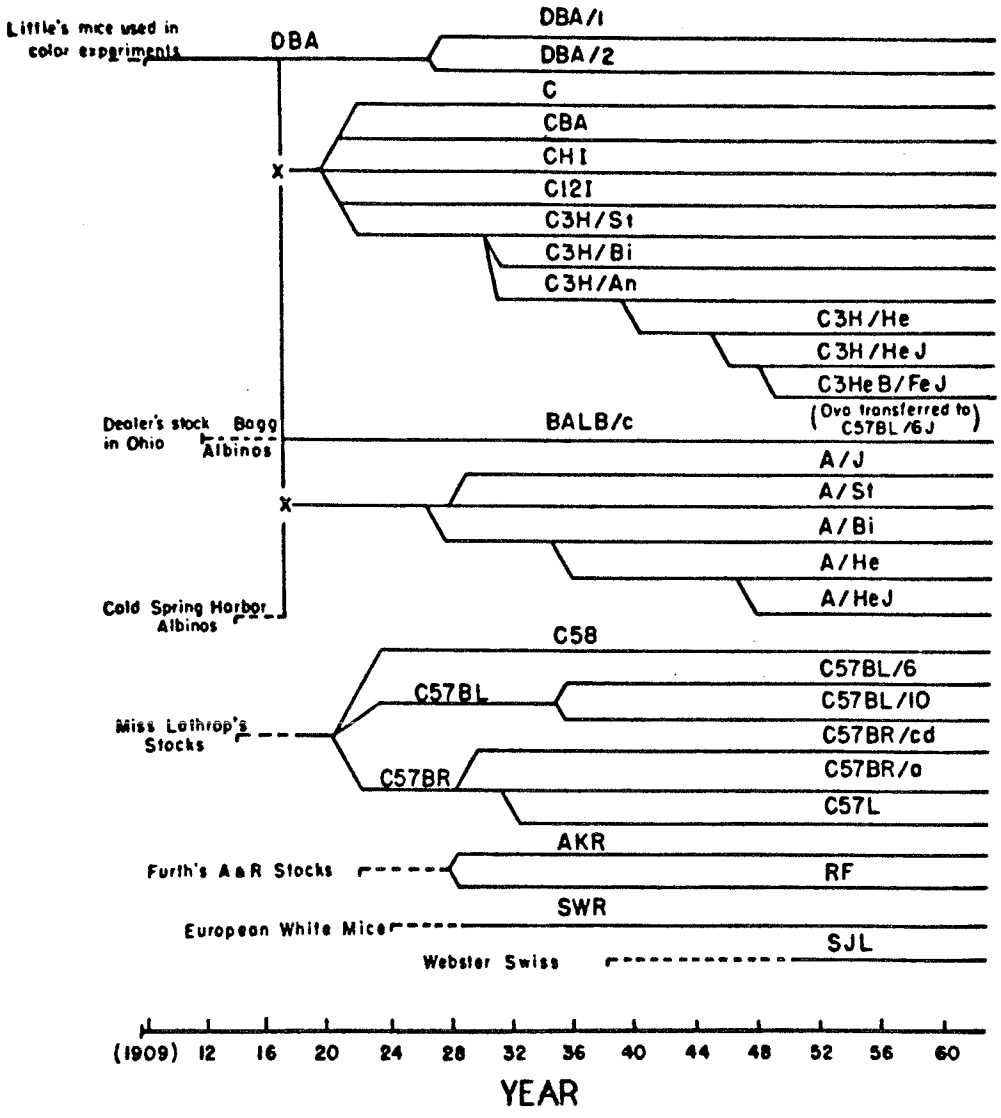


Figure 8: Origins of inbred strains of mice [116].

exploratory experiment was carried out to both establish whether or not cells which mediate contact sensitivity induced IgE responses could be boosted in irradiated recipients, and to determine whether these cells were located primarily in the spleen or the lymph nodes. As outlined in Figures 9 a, b and c, donors of PCl primed cells were "painted" on days -22 and -15 and their spleens and draining lymph nodes (lumbar, caudal, inguinal, axillary, brachial and maxillary) were removed on day -1. Spleen and lymph node cells from both "painted" and "unpainted" donors were then purified by the Ficoll method. Either 3×10^7 of the lymph node or 5×10^7 of the spleen cells were injected into mice which had been given 650 rads of irradiation on the previous day. On the following day (day 0) some of the groups of recipient mice were "painted" with a sensitizing dose of PCl and then boosted 36 days later (day 36). The recipients were bled on days 7, 14, 21, 28, 35 and 43, and the serum IgE titers obtained by PCA. Examination of Table 2 demonstrates that the TNP-specific IgE response to PCl "skin painting" can be transferred to irradiated recipients with 5×10^7 primed spleen cells or 3×10^7 primed lymph node cells. (Unfortunately, because some of the mice were sick and dying, it was not possible to determine titers beyond day 7 for half of the groups.) Recipients of unprimed cells did not demonstrate a response even after a second "painting", which indicated that the above measured responses were produced by the primed donor cells. It should be pointed out that the results of this study should be viewed with caution because they were generated from only one experiment and because some of the mice were sick and dying as a result of the radiation treatment.

D. Boosting TNP-specific IgE Responses to PCl with Haptenated PEC

Evidence suggests that "skin painting" with picryl chloride modifies macrophage-like Langerhan's cells which in turn present TNP in association with surface Ia determinants to cells

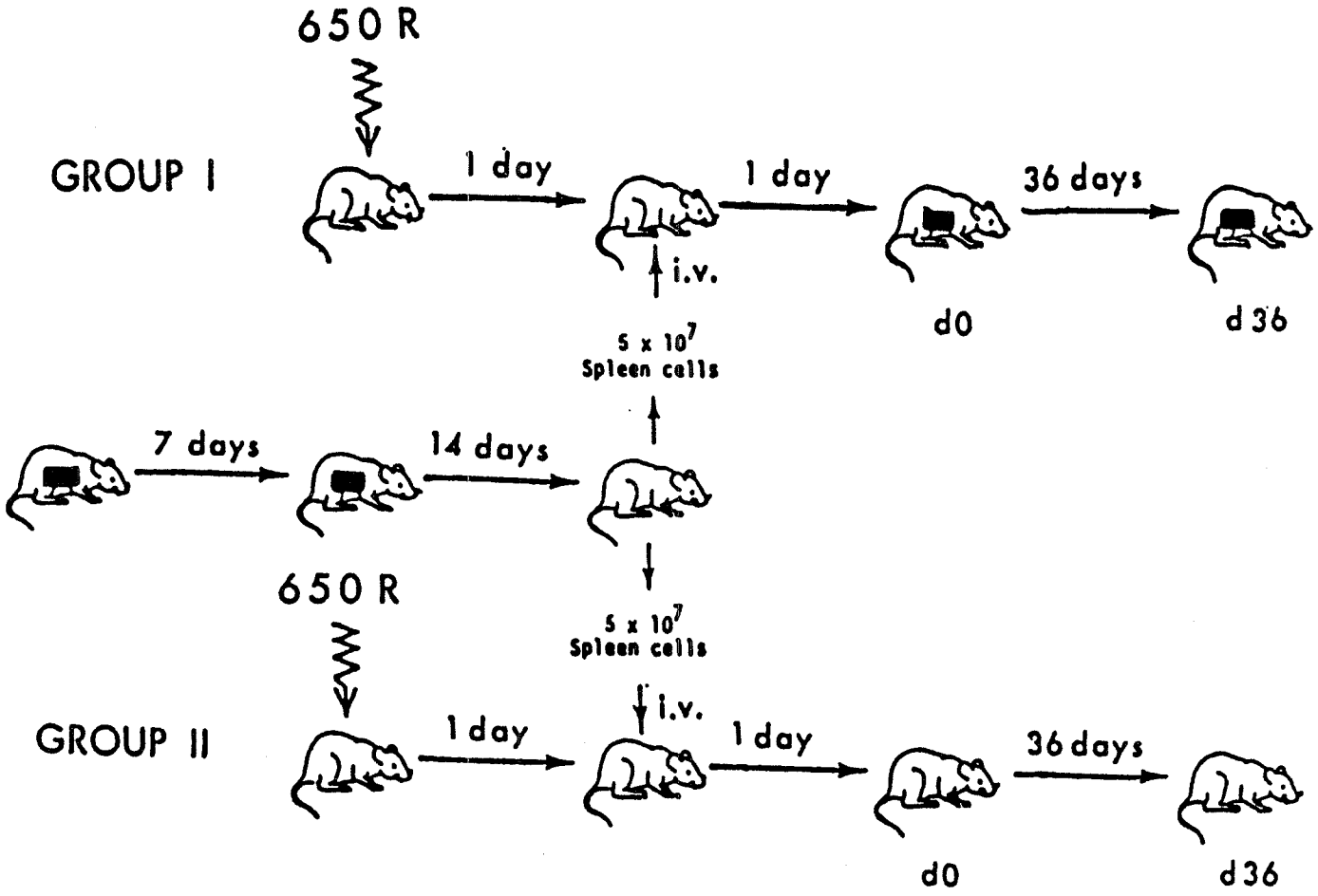

650 R
GROUP I
1 day
1 day
36 days
i.v.
5 x 10^7
Spleen cells
d0
d 36
7 days
14 days
650 R
5 x 10^7
Spleen cells
i.v.
GROUP II
1 day
1 day
36 days
d0
d 36

Figure 9a

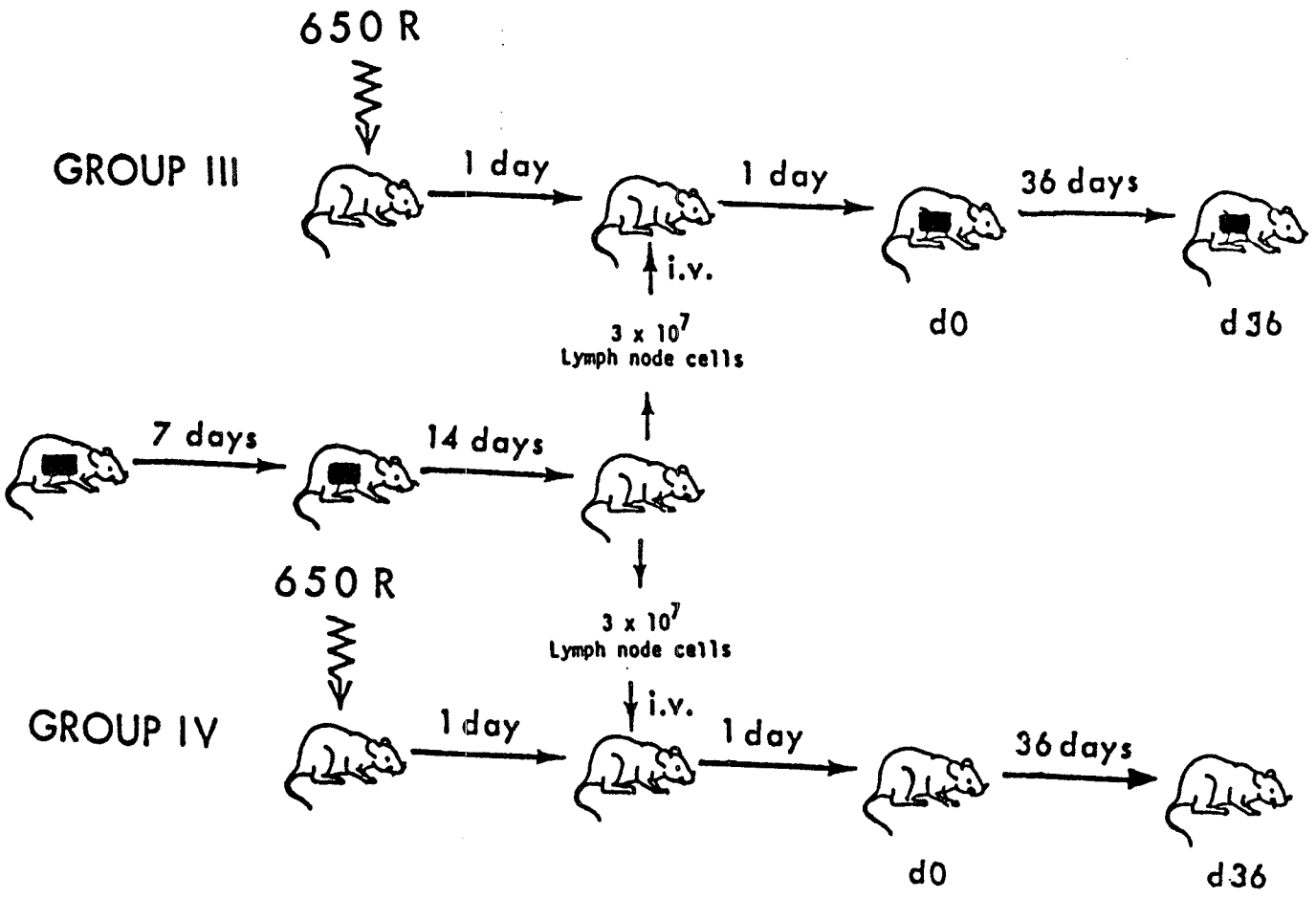

650 R
GROUP III
1 day
1 day
36 days
i.v.
3 x 10^7
Lymph node cells
d0
d36
7 days
14 days
650 R
3 x 10^7
Lymph node cells
i.v.
GROUP IV
1 day
1 day
36 days
d0
d36

Figure 9b

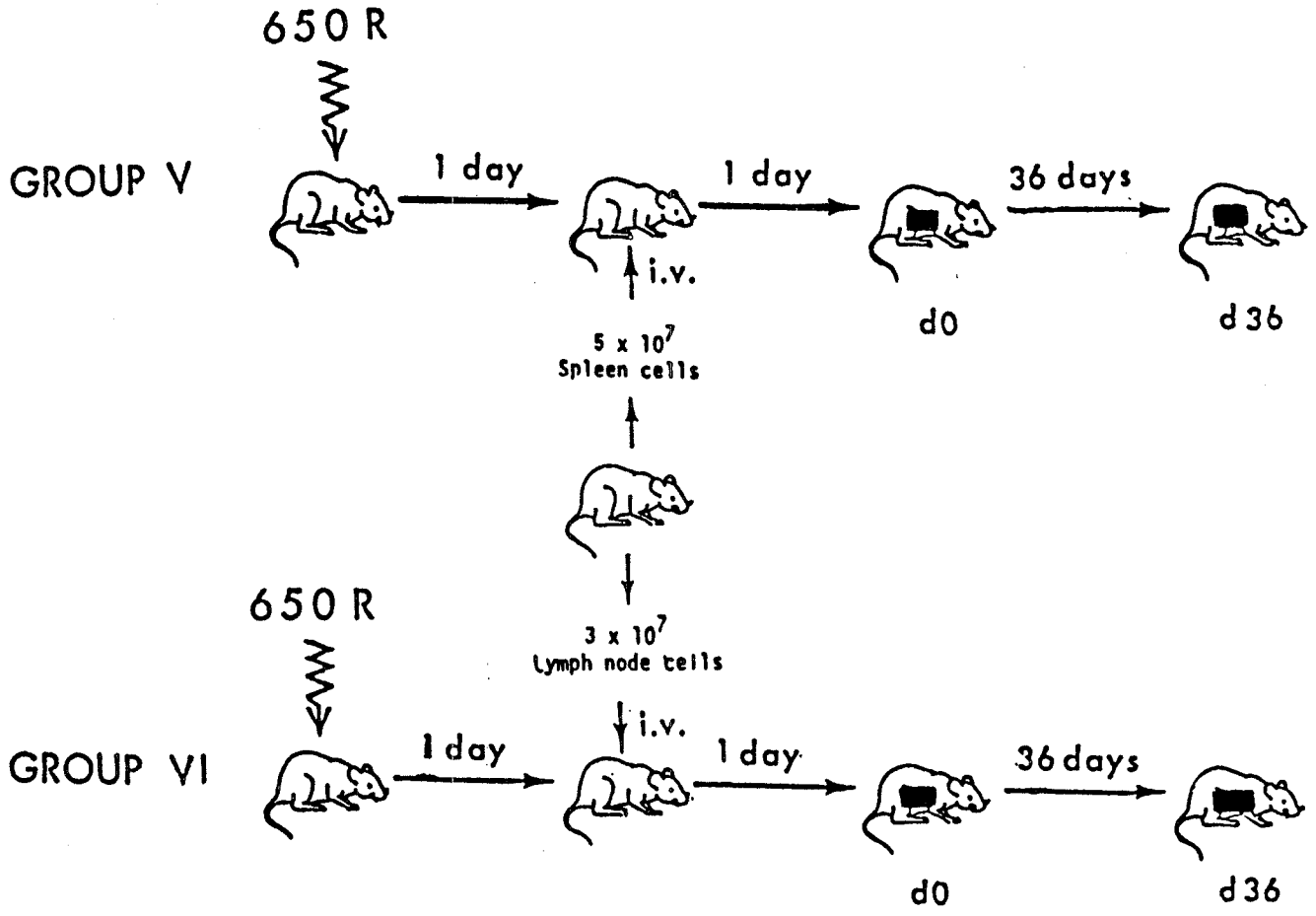

650 R
GROUP V
1 day
1 day
36 days
i.v.
5 x 10^7
Spleen cells
d0
d 36
650 R
3 x 10^7
Lymph node cells
i.v.
GROUP VI
1 day
1 day
36 days
d0
d 36

Figure 9c

Figure 9 (a, b and c): Schematic diagrams of the experimental procedure used to determine if the TNP-specific IgE response could be transferred to irradiated recipients by spleen and/or lymph node cells.

LEGEND

d0----------day 0

d36----------day 36


----------"skin painting"

TABLE 2

Transfer of Contact Sensitization Induced IgE Response to Irradiated CBA/J Recipients

GROUP	Cells Injected i.v. into Irradiated Recipients	PCl Paint of Recipients	TNP-specific PCA Titers on Days					
			7	14	21	28	35	43
I	5×10^7 SC	YES	120	16	16	25	8	50
II	5×10^7 SC	NO	<10	<8	<8	<8	<8	<8
III	3×10^7 LNC	YES	120	ND	ND	ND	ND	ND
IV	3×10^7 LNC	NO	<10	ND	ND	ND	ND	ND
V	5×10^7 SC	YES	<10	<8	<8	<8	<8	<8
VI	3×10^7 LNC	YES	<10	ND	ND	ND	ND	ND

LNC- Lymph node cells

SC-- Spleen cells

which produce IgE [2-3]. To obtain evidence which would either support or impugn this hypothesis, attempts were made to boost the contact sensitization primed cells with TNP-derivatized APC in the absence of additional skin painting. Consequently, CBA/J mice were "painted" on days 0 and 7, and then 5×10^7 of their spleen cells were injected i.v. into previously irradiated (650 rads) syngeneic mice. Subsequently, the recipient mice were injected subcutaneously with 3×10^7 cells of various types (see Figure 10 and Table 3). The recipients were bled 14 days later (day 14) and the PCA titers of the serum samples were determined. As can be seen in Table 3, the responses of all groups which received haptenated PEC (with the exception of TNP-modified non-adherent PEC) were boosted. Neither normal PEC nor haptenated spleen cells evoked significant responses which indicates that boosting required TNP-modified determinants found on PEC. Further, it is interesting to note that only the adherent population was able to boost the TNP-specific PCA response. Hence, it appears that anti-TNP IgE antibody responses in the contact sensitivity response to picryl chloride is directed against a TNP-derivatized macrophage-like cell.

E. Effect Of The Intravenous Injection Of TNP-modified Cells

The i.v. injection of hapten-modified spleen cells has been shown to down-regulate hapten-specific dth and contact sensitization responses by both a direct tolerization of relevent lymphoid cells, and the activation of suppressor T cells [117-118]. Therefore, it was of interest to determine if the TNP-specific IgE response to "skin painting" with picryl chloride would be effected by the i.v. injection of TNP-modified syngeneic cells. As outlined in Figure 11 mice were injected i.v. with varying doses and types of haptenated or normal (see text) syngeneic cells on day -7. Subsequently, all recipient mice were "skin painted" with picryl chloride on days 0 and 7; and, in the case of Experiment 3, also on day 37. The mice

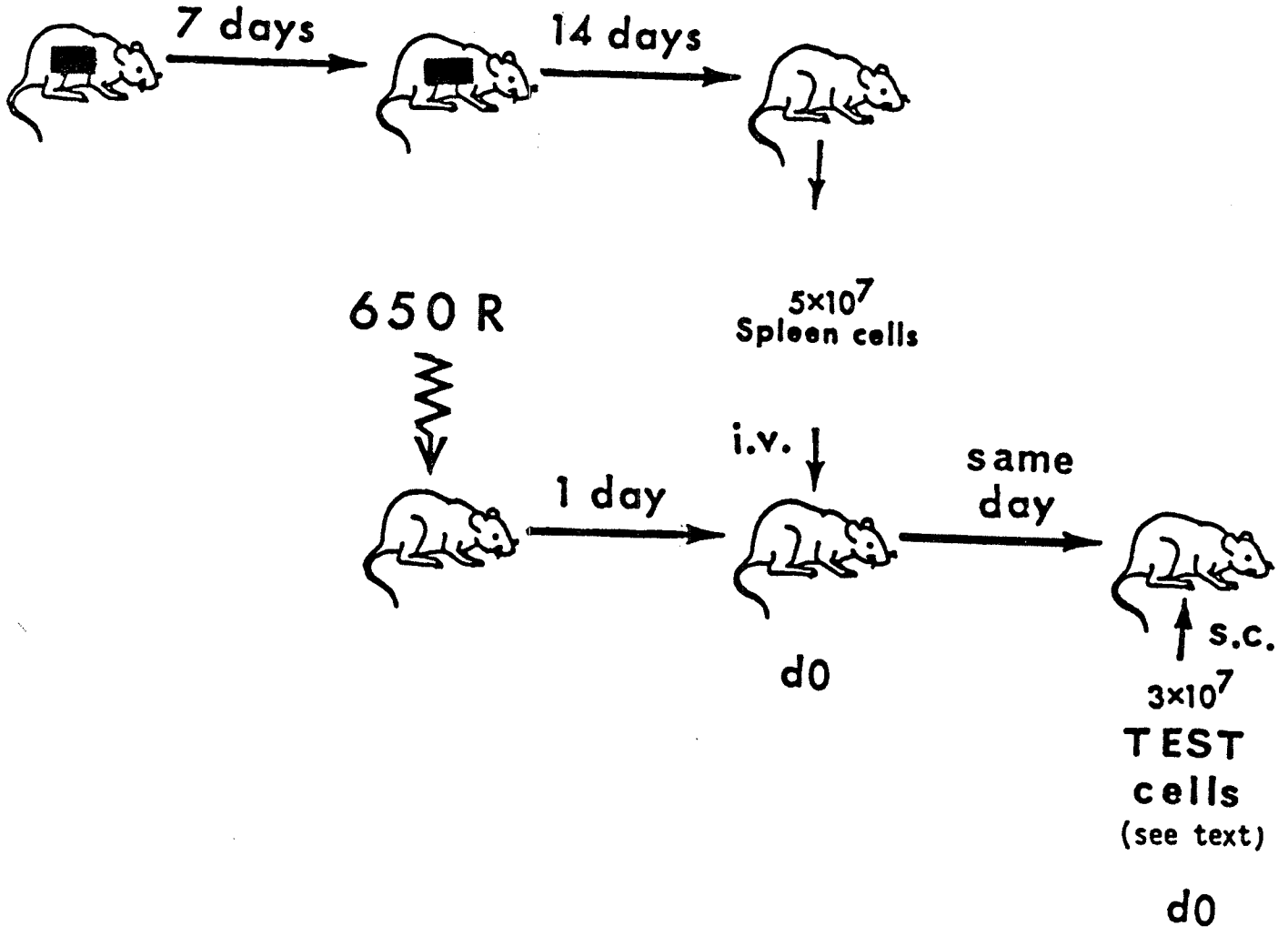

7 days
14 days
650 R
5x10^7
Spleen cells
i.v.
1 day
same
day
s.c.
d0
3x10^7
TEST
cells
(see text)
d0

Figure 10

Figure 10: Schematic diagram of the experimental procedure used to determine if TNP-specific IgE responses were boosted by haptenated PEC.

LEGEND

d0----------day 0

----------"skin painting"

TABLE 3

Boosting TNP-specific IgE Responses to PCl with Haptenated PEC (a)

Experiment	Cells Administered s.c. to Recipients	TNP-specific PCA Titer of Recipient Mice (Day 14)
1	TNP-PEC	25
	PEC	<4
2	TNP-PEC	100
	TNP-Adh-PEC	840
	TNP-SC	6
3	TNP-SC	30
	TNP-Adh-PEC	500
	TNP-non-Adh-PEC	<2

(a) CBA/J mice were painted with the sensitizing dose of PCl on days 0 and 7; on day 21 their spleens were removed and 5×10^7 spleen lymphocytes were transferred i.v. to irradiated (650R), syngeneic recipients. The recipient mice were subsequently injected s.c. in the dorsal flank with 3×10^7 of the indicated TNP-modified cells and 14 days later they were bled and their TNP-specific titers were determined.

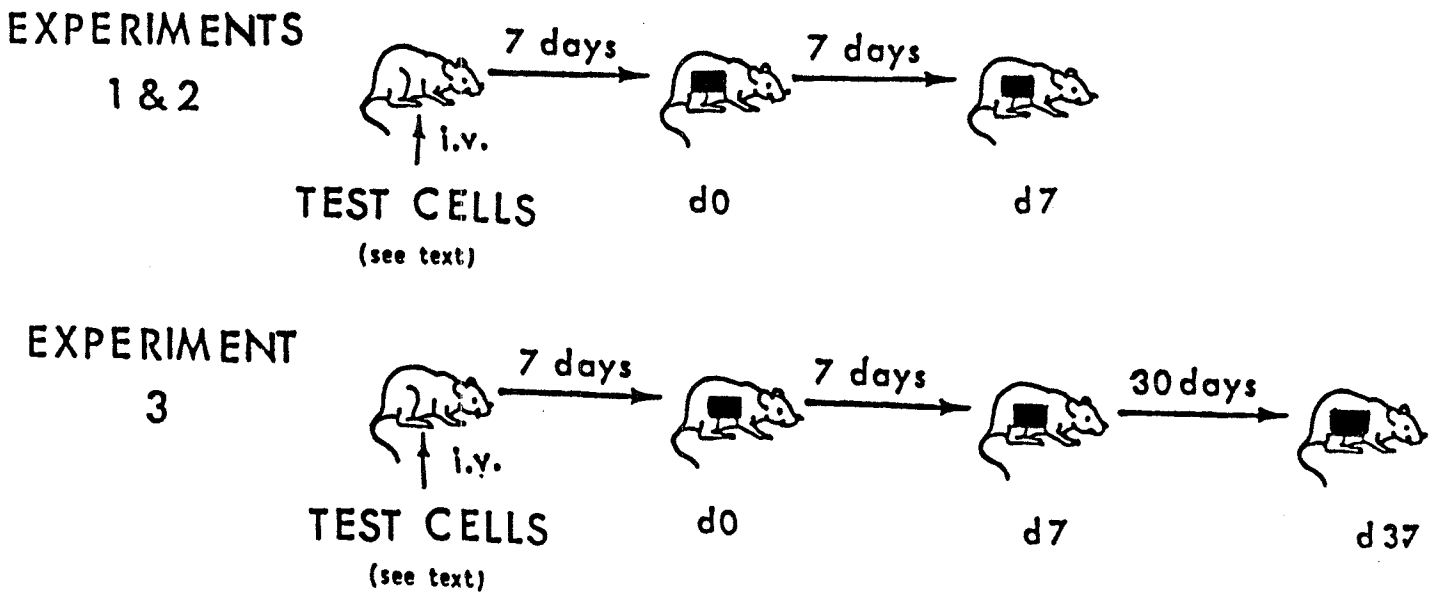

EXPERIMENTS
1 & 2
7 days
7 days
i.v.
TEST CELLS
(see text)
d0
d7
EXPERIMENT
3
7 days
7 days
30 days
i.v.
TEST CELLS
(see text)
d0
d7
d37

Figure 11

Figure 11: Schematic diagram of the experimental procedure used to determine if TNP-specific IgE responses to PCl are suppressed by i.v. injection of haptenated cells.

LEGEND

d0----------day 0

d7----------day 7


----------"skin painting"

were bled on days 14 and 21 (Experiment 1), days 21 and 28 (Experiment 2) and day 37 (Experiment 3). The PCA titers of the sera were obtained in the usual way. Results from Experiments 1 and 2 (see Table 4) disclosed that the injection of TNP-spleen cells reduced the anti-TNP PCA titers of day 14 (Experiment 1) and day 21 (Experiment 2) sera when compared to the groups which received comparable numbers of underivatized (normal) spleen cells (NSC). The fact that animals given TNP-spleen cells in Experiment 1 showed higher day 14 titers that those receiving no cells may indicate that the TNP-spleen cells serve as a form of antigenic stimulation or that some of these cells can also respond to PCl "painting" and therefore augment the response. However, the titers of groups receiving TNP-spleen cells in Experiment 1 were totally abolished by day 14 (PCA titers < 4) in contrast the responses in the NSC treated mice. Interestingly, no dose effect was seen in groups receiving varying numbers of TNP-spleen cells or NSC. Results from Experiment 3 (see Table 4) show that the tertiary day 37 TNP-specific titers were also suppressed in the groups which received TNP-spleen cells compared to those which received normal spleen cells. Also, it can be seen that TNP-modified adherent-PEC quelled the TNP-specific response when injected i.v.

Overall, the results of these experiments were somewhat difficult to interpret because of the unexpected and marked enhancement of the responses of the control groups receiving NSC relative to the responses of the untreated controls.

F. Effect Of Injection Of Normal Spleen Cells

The marked increase in the ability of mice to develop a picryl chloride induced IgE response following the injection of normal spleen cells was an unexpected and interesting finding. Hence additional experiments were carried out to further confirm and characterize this phenomenon. Groups of mice were given 3×10^7 spleen cells from normal donors on days

TABLE 4
Suppression of TNP-specific IgE Responses to PCl with Haptenated Cells (a)

Experiment	Cells Administered (i.v.)	TNP-specific PCA Titer on Day	
		14	21
1	1×10^6 TNP-SC	25	<4
	5×10^6 TNP-SC	55	<4
	5×10^7 TNP-SC	25	<4
	5×10^6 NSC	115	16
	5×10^7 NSC	110	25
	NIL	12	12

		TNP-specific Titer on Day	
		21	28
2	1×10^6 TNP-SC	15	6
	1×10^7 TNP-SC	15	6
	1×10^6 NSC	40	35
	1×10^7 NSC	60	30
	NIL	15	15

		TNP-specific PCA Titer on Day 37
3		
	1×10^7 TNP-SC	140
	5×10^5 TNP-Adh-PEC	110
	1×10^7 NSC	450
	NIL	60

(a) CBA/J mice were injected i.v. on day-7 with varying numbers of the indicated normal or TNP-modified cells and were painted on days 0 and 7 (Experiments 1 and 2) or on days 0, 7 and 30 (Experiment 3) with the sensitizing dose of PCl. The mice were bled at weekly intervals and the TNP-specific PCA titers of the serum samples were determined.

-7, -1 or 6 and were "painted" with PCl on days 14 and 21 (see Figure 12). The results in Table 5 indicate that all NSC injections enhanced the TNP-specific IgE response to some extent and those mice which received cells on days -1 and +6 were significantly higher than control values. Those mice which received NSC just prior to PCl "painting" (day -1) had substantially higher titers than those groups which received cells 7 days prior to or 6 days after the first "skin painting".

G. Effects of Cyclophosphamide On The Contact Sensitization Induced IgE Response

Considerable evidence suggests that cyclophosphamide specifically subdues the effects of Ts cells [119]. Hence, in an attempt to discern the possible role of Ts cells in the TNP-specific IgE response to picryl chloride "painting", mice were injected with cyclophosphamide (Cy) (100 mg/kg body weight) on days -3 and/or +4 and "painted" on days 0 and 7 in two separate experiments. Subsequently, mice were bled on days 14 and 21 and the PCA titers of the serum samples determined (see Figure 13). Somewhat unexpectedly, groups which received single injections of Cy demonstrated slightly lower PCA titers on day 14 than control groups (Table 6). By contrast, mice which were inoculated twice with Cy showed enhanced day 14 responses. The consistancy of the titers in both experiments indicates that two-fold differences are probably significant. However, by day 21 the responses of all groups fell to the level of control groups which did not receive Cy.

H. IgE Synthesis in Response to Contact Sensitization with FITC and NP-O-SUCC

Although this study was principally concerned with the TNP-specific IgE response to "skin painting" with PCl it was also of interest to examine the hapten-specific IgE response

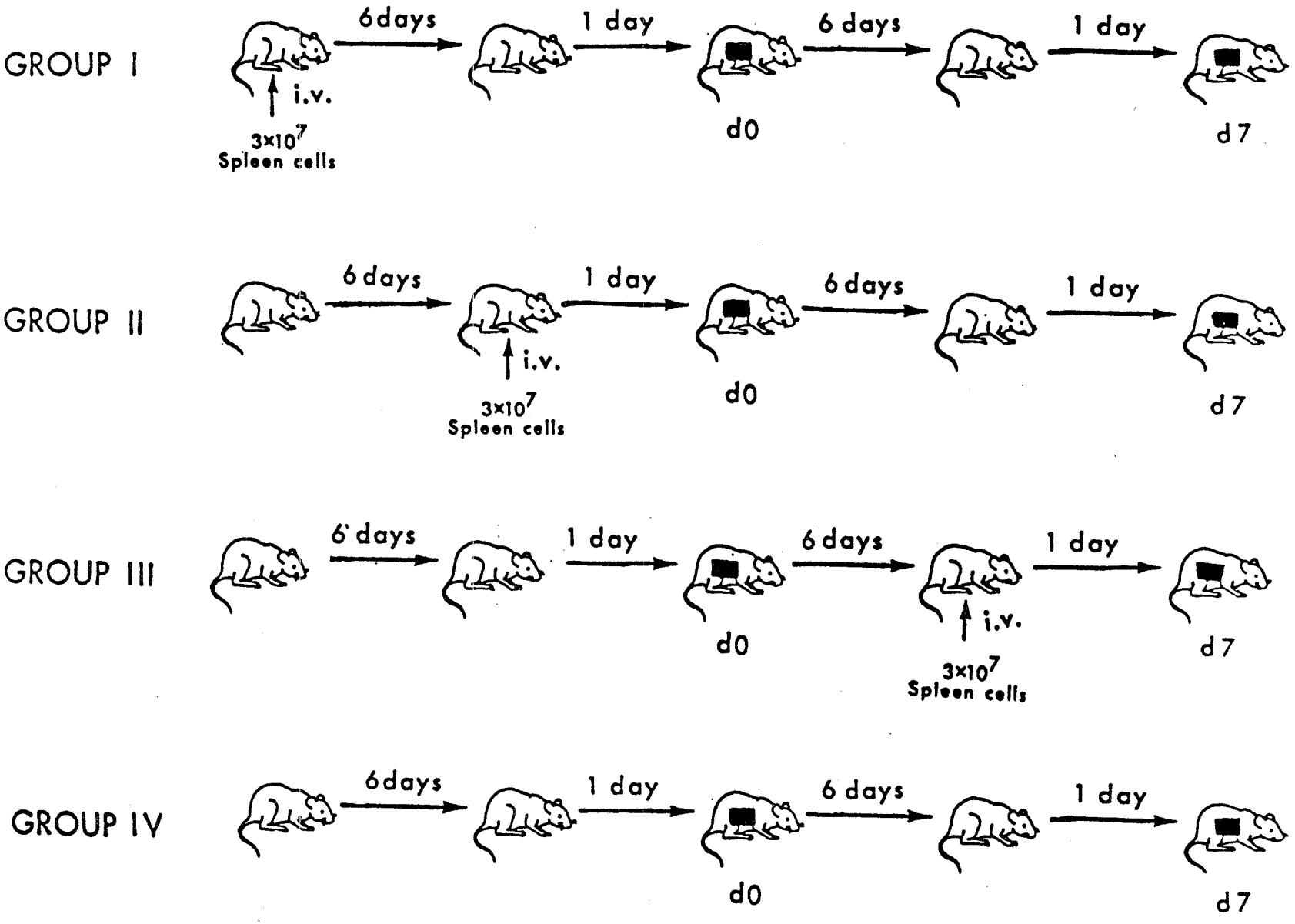

GROUP I
6days
1 day
6 days
1 day
i.v.
3x10^7
Spleen cells
d0
d7
GROUP II
6 days
1 day
6 days
1 day
i.v.
3x10^7
Spleen cells
d0
d7
GROUP III
6 days
1 day
6 days
1 day
d0
i.v.
3x10^7
Spleen cells
d7
GROUP IV
6days
1 day
6 days
1 day
d0
d7

Figure 12

Figure 12: Schematic diagram of the experimental proceedure used to determine the effects of the administration of normal spleen cells (NSC) on the IgE response to PCl.

LEGEND

d0----------day 0

d7----------day 7

---------- "skin painting"

TABLE 5

The Effects of Administration of Normal Spleen Cells on the IgE Response to PCl (a)

Cells Administered (i.v.)	TNP-specific PCA Titer	
	Day 14	Day 21
3×10^7 NSC (day-7)	30	25
3×10^7 NSC (day-1)	130	90
3×10^7 NSC (day+6)	48	16
NIL	12	12

(a) CBA/J mice were administered i.v. on day -7, -1 or +6 3×10^7 normal, syngeneic spleen cells and were painted on days 0 and 7 with the sensitizing dose of PCl. The mice were bled at weekly intervals and the TNP-specific PCA titers of the serum samples were determined.

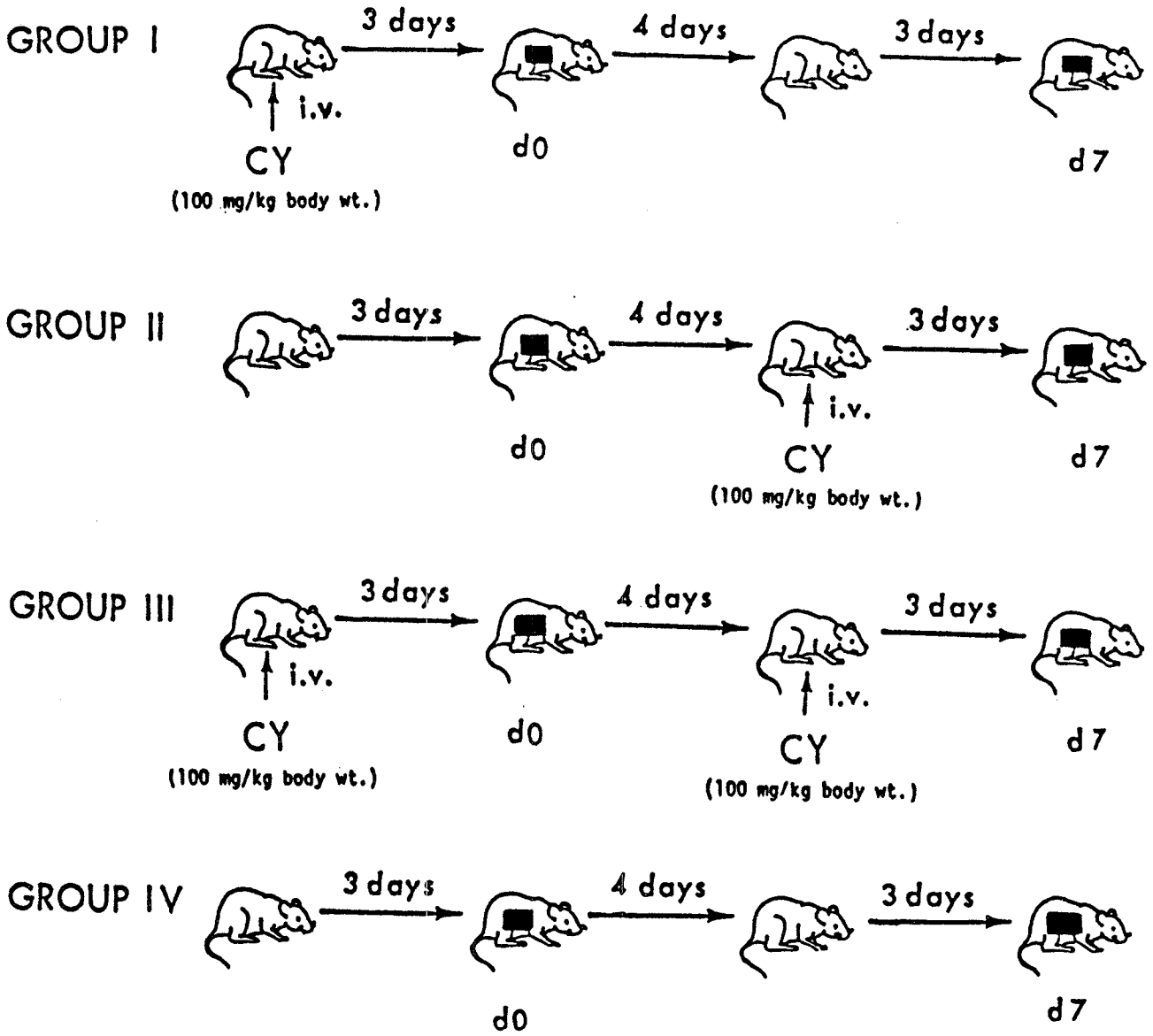

GROUP I
3 days
4 days
3 days
i.v.
CY
(100 mg/kg body wt.)
d0
d7
GROUP II
3 days
4 days
3 days
i.v.
d0
CY
(100 mg/kg body wt.)
d7
GROUP III
3 days
4 days
3 days
i.v.
i.v.
CY
(100 mg/kg body wt.)
d0
CY
(100 mg/kg body wt.)
d7
GROUP IV
3 days
4 days
3 days
d0
d7

Figure 13

Figure13: A schematic diagram of the experimental procedure used to determine the effects of cyclophosphamide on the contact sensitization induced IgE response.

LEGEND

d0----------day 0

d7----------day 7

 ----------"skin painting"

TABLE 6

The Effects of Cyclophosphamide on the Contact Sensitization Induced IgE Response (a)

Experiment	Group	CY Administered i.v.on Days	TNP-specific PCA Titers on Day 14	21
1	I	-3	20	10
	II	+4	10	<4
	III	-3,+4	120	30
	IV	NIL	45	30
2	I	-3	30	25
	II	+4	25	30
	III	-3,+4	110	40
	IV	NIL	35	40

(a) CBA/J mice were injected i.v. with 100 mg. cyclophosphamide per kilogram of body weight on day-3, day+4 or both days and were skin painted with the sensitizing dose of PCl on days 0 and 7. The mice were then bled on days 14 and 21 and their TNP-specific IgE responses were determined

to other skin sensitizing agents. Therefore, various strains of mice were contact sensitized with FITC and NP-O-SUCC on days 0, 21 and 42. Subsequently the mice were bled on days 31 and 47 and the anti-hapten PCA titers of the sera were determined. As can be seen in Table 7, CBA/J (H-2^k) (see Table 8) mice produced a substantial IgE response following contact sensitization with both antigens. All other strains tested, however, with the possible exception of A/J were either non-responders or low responders.

I. Mice Primed with NP-O-SUCC Respond to Challenge with NIP-O-SUCC

Most mice with an $IgC_H{}^b$ allotype have been shown to produce primary NP-specific antibodies which have a higher affinity for NIP than for NP hapten [120-121]. Furthermore, Sunday et al [122] showed that mice with $IgC_H{}^{a,b,d,e\ or\ f}$ allotypes produce an NP-specific contact sensitization response which could be successfully challenged with NIP. By contrast, mice with $IgC_H{}^{c\ or\ j}$ allotypes did not respond to contact sensitivity challenge with NIP [122] following sensitization with NP. It was of interest to determine if the IgE antibody response to the contact sensitizing agents were governed by the same genetic restrictions. Hence, experiments were carried out to test the ability of NIP-O-SUCC to boost the NP-specific IgE response in NP-O-SUCC skin sensitized animals.

C57BL/10 (H-2^b $IgC_H{}^b$) and DBA/2 (H-2^d $IgC_H{}^c$) mice were skin sensitized with NP-O-SUCC on day 0. The mice were then re-sensitized on day 21 with NP-O-SUCC, NIP-O-SUCC or NIL. All groups were bled on days 26 and 31 and the NP-specific PCA titers determined as described in Methods. It is evident from Table 9 that responses produced by C57BL/10 (IgC^{Hb}) and DBA/2 ($IgC_H{}^c$) mice were successfully boosted with NIP-O-SUCC. Hence, from the results of these two experiments NP and NIP seem to

TABLE 7

IgE Synthesis in Response to Contact Sensitization With FITC and NP-O-SUCC (a)

Contact Sensitizer	Strain	Hapten-specific PCA Titers on Days	
		31	47
FITC	CBA	90	500
	B10.A	<4	<4
	B10	6	10
	DBA/2	<4	<4
	B6D2F1	<4	<4
NP-O-SUCC	CBA	10	180
	A/J	25	ND
	B10.A	<4	4
	B10	20	35
	DBA/2	6	10
	B6D2F1	6	35

(a) Mice were injected s.c. on days 0,21 and 42 with either FITC or NP-O-SUCC, as described under METHODS, and were bled on days 31 and 47. The hapten-specific PCA titers of the serum samples were then determined.

TABLE 8

H-2 Haplotype of Various Strains of Mice Immunized with FITC and NP-O-SUCC

Strain	H-2 Haplotype						
	K	Aβ	Aα	Eβ	Eα	S	D
CBA ($H\text{-}2^k$)	k	k	k	k	k	k	k
B10.A ($H\text{-}2^a$)	k	k	k	k	k	d	d
B10 ($H\text{-}2^b$)	b	b	b	b	b	b	b
DBA/2 ($H\text{-}2^d$)	d	d	d	d	d	d	d
A/J ($H\text{-}2^a$)	k	k	k	k	k	d	d
B6D2F1 ($H\text{-}2^{b/d}$)	b/d	b/d	b/d	b/d	b/d	b/d	b/d

TABLE 9

Mice Primed With NP-O-SUCC Respond to Challenge With NIP-O-SUCC[(a)]

Experiment	Strain	Contact Sensitizer Administered on Day 0	Contact Sensitizer Administered on Day 21	Anti-NP PCA Titer on Day 26	Anti-NP PCA Titer on Day 31
1	C57BL/10	NP-O-SUCC	NP-O-SUCC	30	5
		NP-O-SUCC	NIP-O-SUCC	5	20
	DBA/2	NP-O-SUCC	NP-O-SUCC	<4	10
		NP-O-SUCC	NIP-O-SUCC	<4	10
2	C57BL/10	NP-O-SUCC	NP-O-SUCC	15	15
		NP-O-SUCC	NIP-O-SUCC	<4	20
		NP-O-SUCC	NIL	<4	<4
	DBA/2	NP-O-SUCC	NP-O-SUCC	25	10
		NP-O-SUCC	NIP-O-SUCC	10	5
		NP-O-SUCC	NIL	<4	<4

(a) Mice were administered s.c. injections of NP-O-SUCC on day 0 and of either NP-O-SUCC, NIP-O-SUCC or NIL on day 21. The mice were bled 5 and 10 days after the second injection and the anti-NP PCA titers of the serum samples were determined.

cross-react in the IgE response to contact sensitizing agents. In this respect, the IgE antibody response to skin sensitization with NP appears to differ from the NP-specific contact sensitization induced inflammation response. However, these conclusions should be viewed with some caution due to the fact that the titers obtained were very low and variable, possibly because of the method of sensitization.

DISCUSSION

I. The Genetics of the Contact Sensitivity Response

Various studies have shown that the contact sensitivity responses to picryl chloride [123-124] and oxazolone [125] are heavily influenced by the genes within the MHC. T cells which can adoptively transfer the contact sensitivity response (T_{CS}) are restricted to K and/or D determinants [14]. Hence it would be expected that the haplotype of the K and/or D subregions of the MHC would play an essential role in the induction of contact sensitivity. Accordingly, Schultz and Bailey [123] found that the haplotypes of both the K end (K I-A) and the D end of the MHC greatly influence the level of contact sensitivity to picryl chloride. More specifically, they proposed that K^d and D^k haplotypes were associated with high responses while K^k, D^d, K^b and D^b haplotypes foretold of weak contact sensitivity responses [123]. A subsequent study by Thomas et al [124] confirmed that B10 ($H\text{-}2^b$) mice, which possess K^b and D^b haplotypes, produced poor contact sensitivity responses to "skin painting" with picryl chloride. However, in contrast to the earlier study [123], Thomas et al [124] established that B10.A ($H\text{-}2^a$) mice, which possess the K^k and D^d haplotypes, responded as well as B10.D2 ($H\text{-}2^d$) and B10.BR ($H\text{-}2^k$) mice which have K^dD^d and K^kD^k haplotypes respectively. In view of these differences, the aforementioned studies of Schultz and Bailey might be reinterpreted and better explained by suggesting that mice possessing K I-A and/or D regions of the MHC with the "b" haplotype are low responders. By contrast, mice for which both the K I-A and D regions are of "k" or "d" haplotypes produce a strong response.

Genes outside the MHC (background genes) also play a large role in determining the level of contact sensitivity responses to various compounds [123-125]. For instance, mice with C58/J, C57/J or A/WySn backgrounds produced strong contact sensitivity responses to

picryl chloride while mice with C57BL/10Sn or C3H.HeJ backgrounds produce poor responses [123]. The same study established that while gender was not a factor, age did play a role in determining the ability of a mouse to generate a contact sensitivity response to picryl chloride. Thus, Balb/c mice were able to produce maximal responses at 70 days of age but only control responses by 185 days of age.

II. The Genetics of the Antibody Response to Contact Sensitizing Agents

Genes within the MHC [123-125] extensively influence the humoral responses to contact sensitizing agents. Thomas et al [124] examined the IgE and IgG responses to "skin painting" with picryl chloride in congenic mouse strains which shared a common B10 background. These conditions would appear to be ideal for studying the effects of the H-2 haplotype upon the IgG and IgE responses. However, results from the present study indicated that strains with a B10 background produce poor IgE responses to contact sensitizing agents. Nevertheless, Thomas et al [124] found that B10 ($H-2^b$) mice were poor IgE and IgG responders, while B10.BR ($H-2^k$) mice were capable of strong antibody responses of both classes. Thomas et al [124] also discovered that B10.A ($H-2^a$) and B10.D2 ($H-2^d$) strains produce strong IgG responses in conjuction with poor IgE responses. As no examination of the IgG response was made in the present study, their conclusions about IgG could not be supported, or refuted, on the basis of the present results. However, it can be seen in Table 1 that in this study B10.A ($H-2^a$) and DBA/2 ($H-2^d$) mice are not strong TNP-specific IgE responders to picryl chloride. Other studies [126-127] have found that AQR ($H-2^{y1}$) mice can generate strong IgE and weak IgG responses simultaneously. In this study AQR mice were similarly seen to demonstrate a strong TNP-specific IgE response after two "skin paintings" with picryl chloride (see Table 1). In addition, this study also demonstrated that H-$2^{a,k,s \text{ and } q}$ haplotypes favor the TNP-specific IgE response to skin painting with picryl chloride. Hence, it can be concluded that the results of this study are in general agreement with the conclusions of the preceding literature. Moreover, the present study was the first to demonstrate that mice with H-$2^{s \text{ or } q}$ haplotypes tend to produce high TNP-specific IgE responses to "skin painting" with picryl chloride. In view of the fact that cells of the H-$2^{q \text{ or } s}$ haplotypes do not express I-E encoded determinants and that there is now some agreement that many Ts cells are restricted by I-E determinants, the results tend to suggest a dominant role for Ts cells in the regulation of these

IgE responses. By comparing the TNP-specific responses of mice with identical MHC haplotype, but with different backgrounds, {eg. C3H ($H\text{-}2^k$) vs B10.BR ($H\text{-}2^k$); A/J ($H\text{-}2^{k/d}$) vs B10.A ($H\text{-}2^{k/d}$); A.SW ($H\text{-}2^s$) vs B10.S ($H\text{-}2^s$)} (see Figure 8)this present study was also the first to show that non-MHC genes influence the TNP-specific IgE response to picryl chloride. It was also seen that the "so-called" IgE non-responder strain AKR produced a poor response to picryl chloride despite having a $H\text{-}2^k$ haplotype, thus paralleling its IgE response to protein conjugates.

An exploratory examination of the hapten-specific IgE responses to contact sensitization with FITC and NP-O-SUCC was also undertaken. As shown in Table 7, CBA/J ($H\text{-}2^k$) produce a substantial IgE response following contact sensitization with both antigens. All other strains, with the possible exception of A/J were, however, either non-responders or low-responders. It would perhaps be not too meaningful to compare the ability of the various strains to produce IgE antibodies in response to picryl chloride vs FITC and NP-O-SUCC because of the different methods of skin sensitization that were used in these experiments. It is, however, likely safe to conclude that CBA/J mice are strong IgE responders to contact sensitization with all three antigens and that this strain is, therefore, the most appropriate strain for the study of hapten-specific IgE responses to various contact sensitizing agents.

III. The Fine Specificity of the Antibody Response to Contact Sensitizing Agents

It would be of interest to know if the concomitant contact sensitivity and IgE antibody responses to contact sensitizing agents are mediated and/or helped by T cells bearing identical antigen receptors. Thermodynamic laws would seem to favor the expansion of clones which bear receptors with the highest affinity for a particular hapten [128]. In some cases, a response is generated which involves antibody and/or receptors which bind an antigen distinct from the immunogen with a greater affinity than they bind the immunogen. For example mice with an $IgC_H{}^b$ allotype produce primary antibodies to NP which bind NIP with a higher affinity than they bind NP [120-121]. In a somewhat controvertial study, Sunday et al [122] claimed that the contact sensitivity induced inflammatory response to NP-O-SUCC could be boosted by NIP-O-SUCC in mouse strains with $IgC_H{}^{a,b,d,e \text{ and } f}$ allotypes but not in mice with $IgC_H{}^{c \text{ and } j}$ allotypes. This present study also examined the ability of NIP-O-SUCC to boost the NP-O-SUCC-generated NP-specific IgE response in C57BL/10 ($IgC_H{}^b$) and DBA/2 ($IgC_H{}^c$) mice. As can be seen in Table 9 the NP-specific IgE response to NP-O-SUCC could be boosted by NIP-O-SUCC in both the C57BL/10 ($IgC_H{}^b$) and DBA/2 ($IgC_H{}^c$) mice. Hence, it is suggested that in DBA/2 mice the IgE antibody response to NP-O-SUCC is mediated by a repertoire of lymphocytes which are distinct from those which mediate the concomitant contact sensitivity induced inflammatory response. It is possible that the cells mediating the IgE response produced by DBA/2 in the present study may not be heteroclitic but rather they may only recognize NIP. However, due to the low titers generated in this experiment the results and conclusions drawn from them should be viewed with caution. Further experiments in which the IgE and inflammatory responses are measured simultaneously would be required to substantiate these hypotheses.

IV. The Regulation of the Antibody Response to Contact Sensitization

A. Induction of the Response

It appears that the contact sensitivity inflammatory response is induced by antigen modified MHC determinants on "macrophage-like" Langerhan's cells [2-3]. In an attempt to characterize the APC involved in the TNP-specific IgE response to picryl chloride "skin painting", various cell populations were haptenated with TNP and their ability to boost the TNP-specific IgE response in syngeneic irradiated recipients was assessed. As can be seen in Table 3 (Experiments 1 and 2) haptenated PEC were able to significantly boost the response, while TNP-SC and normal (unhaptenated) PEC were not. A closer examination of the adherent and non-adherent PEC populations (Experiment 3) revealed that the "macrophage-like" adherent PEC were responsible for boosting the response. This would indicate that the humoral and contact sensitivity responses to picryl chloride can be boosted by similar, if not identical, cells. Further work with TNP-modified Langerhan's cells and other "macrophage-like" cell populations will be needed to resolve this question. It would also be interesting to discover not only which cells present antigen (APC) but the actual surface determinants involved. This might require attempts to boost the TNP-specific IgE response with haptenated APCs from congenic mice bearing recombinant H-2 haplotypes.

B. Effector and Helper Cells of the Response

Cells which can transfer dth to naive recipients are found in the spleen and draining lymph nodes [129], those which generate primary TNP-specific primary PFC responses to picryl chloride "skin painting" are located in the spleen and not the lymph nodes that drain the innoculation site [130] 2-3 days after skin sensitization. It seems from this study (Table 2) that the cell responsible for the concomitant IgE response is also located in both the spleen and

draining lymph nodes. This similarity between the location of the cells which mediate the dth and humoral responses adds further evidence that the two responses are mediated by similar subpopulations of T cells. As outlined in the Results section, however, these conclusions should be considered tentative, since the results were from only one experiment and since some of the mice were sick and dying as a result of the radiation treatment.

C. Class and Antigen-specific Suppressor Cells

There are both class and antigen-specific T suppressor cells which regulate the TNP-specific antibody response to "skin-painting" with picryl chloride. However, like the T suppressor cells involved in the suppression of TNP-specific contact sensitivity, the various populations of humoral-acting Ts cells have not been well characterized. While an examination of all these cells was beyond the scope of this study, some preliminary examination of the role of Ts cells was made.

Miller et al [117] demonstrated previously that the i.v. injection of DNP-derivatized spleen cells (DNP-SC) into mice markedly inhibited the ability of these animals to develop a DNP-specific, contact sensitization induced inflammatory response. The mechanisms underlying this phenomenon appeared to involve both the direct tolerization of appropriate lymphoid cells, and the activation of Ts cells [118]. Therefore, it was of interest in the present study to determine whether or not the IgE response to skin painting with PCl could also be suppressed by the i.v. injection of TNP-SC. As shown in Table 4, mice receiving as few as 1×10^6 TNP-SC i.v. 7 days prior to skin sensitization developed a notably reduced TNP-specific IgE response relative to that produced by control mice receiving 5 to 50×10^6 NSC. The interpretation of these results was, however, somewhat complicated by the observation that the above control mice developed an enhanced IgE response

relative to that produced by a second group of control animals which had not been injected with SC prior to skin sensitization. The PCA titers of day 21/28 sera from the test mice in Experiments 1 and 2 were lower than the titers of the corresponding sera from the untreated controls, but the degree of suppression was, of course, less than when the former titers were compared to those of the sera obtained from NSC treated controls. Overall, these results could be cautiously interpreted to indicate that the i.v. injection of TNP-SC could inhibit the ability of mice to elaborate a contact sensitization provoked IgE response. Whether or not this suppression was due, in part, to the activation of Ts cells awaits further investigation.

The enhancement of the PCl induced IgE response following the i.v. injection of NSC was an unexpected but interesting finding. The mechanism(s) underlying this enhancement are currently not clear, but the result may simply have been due to an increased number of hapten reactive lymphoid cells in the responding animal. It would be of interest to repeat these experiments using subpopulations of NSC in order to further elucidate the mechanism.

Further evidence for the presence of T suppressor cells in the TNP-specific IgE response to PCl came from the examination, in this study, of the effect of cyclophosphamide on the response. It is known that cyclophosphamide can selectively abolish T suppressor cell activity [74,119,131]. As can be seen in Table 6, groups which received cyclophosphamide (375 mg/kg body weight) three days before both the first and the second picryl chloride "painting" demonstrated enhanced TNP-specific IgE titers. By contrast, groups which received one cyclophosphamide treatment (either before or the first or the second picryl chloride "painting") showed titers which were not increased relative to those of control groups. Hence, this study has presented additional albeit very suggestive evidence that the TNP-specific IgE response to picryl chloride "skin painting" is under T suppressor cell control.

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