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EDITORIAL REVIEW

Yes T cells, but three different T cells ($\alpha\beta$, $\gamma\delta$ and NK T cells), and also B-1 cells mediate contact sensitivity

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SUMMARY

Transfer of contact sensitivity (CS) responses by immune lymphoid cells was the first finding that distinguished cellular from humoral immunity. CS has remained the most studied T cell reaction *in vivo*, and is the prototype for a variety of delayed-type hypersensitivity (DTH) responses. DTH in essence is the recruitment of effector αβ-T cells out of vessels into peripheral tissues. The T cells then are activated by antigen presenting cells to produce pro-inflammatory cytokines. It has been assumed that the αβ-T cells alone are responsible, but recent studies show that three other lymphocyte subsets are involved: CS-inducing NK T cells, CS-initiating B-1 cells, and CS-assisting γδ-T cells. Therefore, the effector αβ-T cells are essential, but cannot be recruited into the tissues without the local action of IgM antibodies produced by B-1 cells rapidly (1 day) post-immunization. The IgM complexes with the challenge antigen to locally activate complement to lead to vascular activation required for T cell recruitment. This process occurs early (1-2 hours) in the elicitation phase, and is called CS-initiation. The essential CS-inducing NK T cells activate the B-1 cells by producing IL-4 rapidly (1 hour) after immunization, and γδ-T cells assist the local inflammatory function of the recruited CS-effector αβ-T cells. Thus, four lymphocyte subsets are required for elicitation of responses: CS-inducing NK T cells, CS-initiating B-1 cells, CS-assisting γδ-T cells, and finally the CS-effector αβ-T cells. Three of these four cell types are present in the immune lymphoid cell population that adoptively transfers CS: B-1 cells, γδ-T cells, and the αβ-T cells.

Keywords contact hypersensitivity $\gamma\delta$ T cells $\alpha\beta$ T cells NK T cells

INTRODUCTION

Contact sensitivity (CS) is the oldest and most frequently studied form of *in vivo* T cell mediated immunity. Experiments in the 1940s first established the concept of cellular immunity by transfer of CS responses with immune cells, and not by serum antibodies [1]. Until recently, the paradigm for these prototypic *in vivo* cell mediated immune responses was that they were mediated by sensitized effector $\alpha\beta$-T cells recruited into the tissues to bind antigen peptides complexed with MHC molecules on the surface of local antigen-presenting cells. This cognate recognition caused T cell activation for production of cytokines like IFN-γ, to orchestrate local inflammation featuring perivascular infiltrates of mononuclear cells [1]. Lately, there has been an explosion of new knowledge about CS that overturns this simple conception. These findings indicate that yes, T cells are essential, but in fact three different kinds of T cells; namely the CS-effector $\alpha\beta$-T cells [1], but also CS-assisting $\gamma\delta$-T cells [2], and interesting and very recently, CS-inducing NK (natural killer) T cells are required [3]. Finally, and perhaps most surprising for responses defined as free of B cells and antibodies, it has been found that *CS*-initiating B-1 cells producing IgM antibody also are required (unpublished observation). A paper in the current issue by Yokozeli *et al*. [4] confirms the involvement of CS-assisting $\gamma\delta$ T cells. This review will place these data in the context of the four different specific cells that are all necessary for the induction and elicitation of CS responses.

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CS-effector $\alpha\beta$-T cells and Langerhans cells

Induction of CS begins with skin painting contact immunization employing simple reactive chemicals, such as picryl chloride (PCl), oxazolone (OX), or DNFB in the laboratory, or poison ivy, nickel, chromium and many other substances in the clinic. These chemicals bind, often covalently, to the terminal amino group on lysines in host skin proteins to constitute neo-antigens that are processed in skin Langerhans presenting cells (APC) into hapten-self peptides that complex with surface MHC and are borne to draining lymph nodes by the APC. Then the Langerhans cells differentiate into mature dendritic cells that activate recirculating T cells that have specific $\alpha\beta$-TCR appropriate for binding the hapten-peptide-MHC complex. By day 4, this results in activation

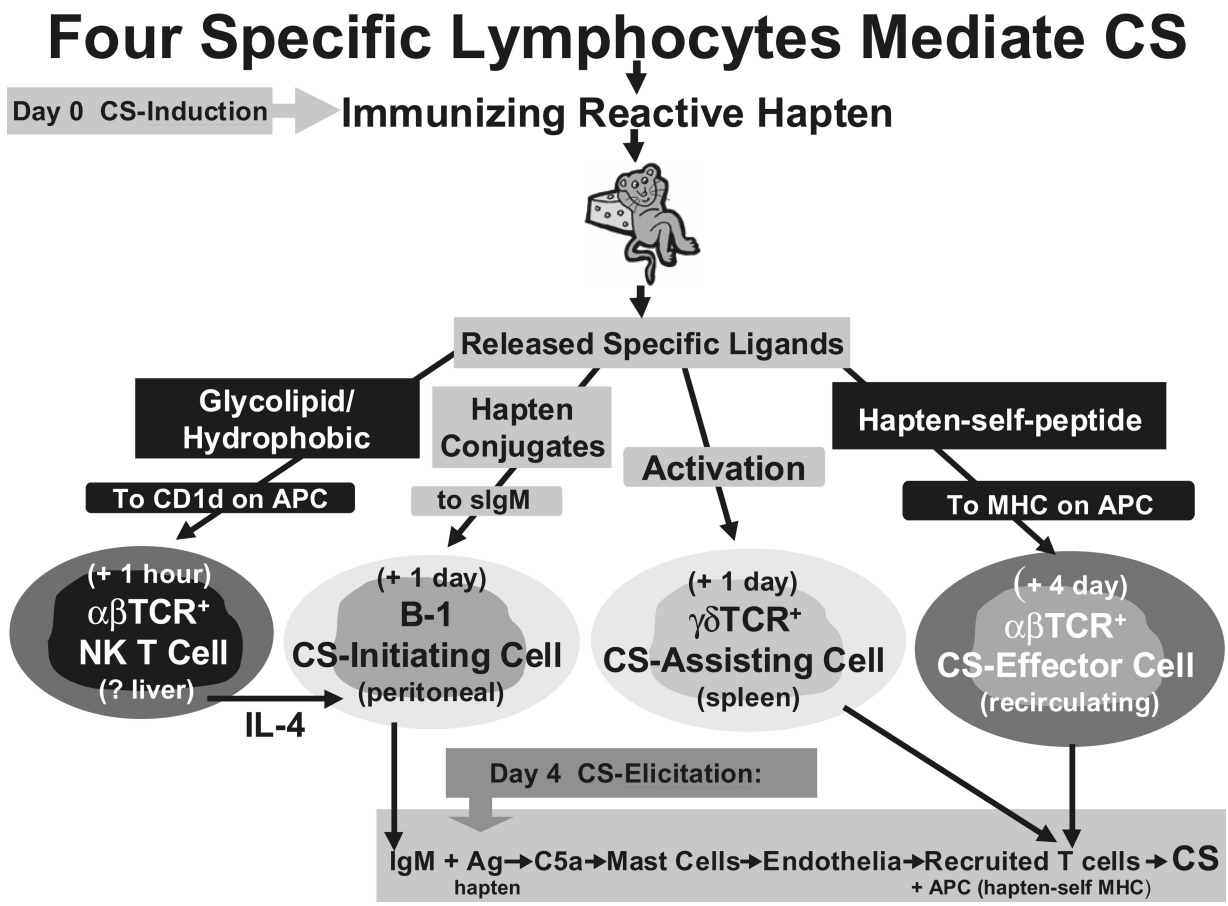

Fig. 1. The lymphocytes involved in CS. At priming for induction of CS (i.e. immunization), released glycolipid ligands activate the first-acting T cells via their unusual semi-invariant αβ-TCR. These are CS-inducing NK T cells that provide crucial IL-4 to allow coactivation of the second-acting CS-initiating B-1 cells in the peritoneal cavity, along with systemically dispersed hapten-self protein conjugate Ag. This stimulates the B-1 cells to produce specific IgM antibodies that enter the circulation. This priming sensitization also activates and mobilizes the third acting CS-assisting γδ-T cells from the spleen. Finally, CS-effector αβ-T cells are activated in the lymph nodes by Ag-MHC-APC on dendritic APC, as the fourth and final effector cells of CS.Then, at secondary hapten Ag challenge for elicitation of CS, the locally available B-1 cell-derived IgM binds Ag to lead to an early Ag-dependent local CS-initiating processes that generates C5a, to locally recruit the CS-effector αβ-T cells, that are aided by corecruited CS-assisting γδ-T cells for optimal activation by the APCs to produce cytokines that mediate CS.

to become sensitized CS-effector T cells, which again recirculate to be able to mediate the late classical 24 h component of CS if recruited into the tissues [1], but actually are the fourth-acting lymphocyte subset in CS (Fig. 1).

CS-inducing NK T cells

Recent work indicates that $\alpha\beta$-T cells are necessary but not sufficient for the subsequent elicitation of CS responses. Three other classes of lymphocytes with specific receptors also are required. During the first day following sensitization important separate processes are triggered by contact skin painting that activate the other lymphocytes needed to bring about the final elicitation of CS. Amazingly, NK T cells are activated within the first hour following sensitization to become the first-acting required lymphocyte subset in CS. NK T cells were discovered only 14 years ago and have been shown to mediate several regulatory functions in other systems [5]. They have NK cell markers and receptors, but also express $\alpha\beta$-TCR that however, are semi-invariant. In mice, most NK T cells express a single TCR-α chain of Vα14, Jα281 that is paired with either of 3 Vβ chains. This canonical $\alpha\beta$-TCR of the dominant NK T cells preferentially recognizes glycolipids, like α-Galactosyl-Ceramide (α-Gal Cer), derived from marine sponges, and also binds some hydrophobic peptides [6]. Lipid binding usually is in the context of the MHC class I-like molecule CD1d on APC that has a deep lipid-binding hydrophobic groove [6]. The complex of glycolipid and CD1d activates NK T cell V$\alpha14^+$ TCR causing rapid and strong production of several cytokines, mostly IL-4 and/or IFN-γ [5,6].

The location of NK T cells activated very early in CS is not known. The major population residing in the liver or bone marrow [5,6], or minor numbers in the peritoneal cavity, might be involved. Contact sensitivity causes immediate systemic dispersion of the sensitizing hapten from the skin [7], and presumably hapten-protein and peptide complexes. Since there likely is release of other materials, we postulate that distant NK T cells are activated by hydrophobic, substances such as endogenous glycolipids that are released from the skin in the first hour following contact sensitization, perhaps as 'danger' signals

emanating from damaged skin cells [8], to alert cytokine-producing NK T cells that express semi-invariant glycolipid-recognizing $\alpha\beta$-TCR [6]. A crucial effect of this NK T cell activation early in CS is the production of IL-4 [3], and likely other cytokines [5]. NK T cell-derived IL-4 has been demonstrated in the circulation within the first day of contact sensitization [9], and also rapidly following NK T cell activation by injection of anti-CD3 [10].

CS-initiating B-1 cells

The rapidly released NK T cell-derived IL-4 has a crucial role in the activation of a particular specific B cell subset that is required to elicit CS. This also occurs within the first hour following sensitization and depends on the NK T cells [3]. These are B-1 cells that reside mainly in the peritoneal cavity and the second-acting lymphocyte subset required to elicit CS. The B-1 cells produce IgM antibody to trigger a process that rapidly follows elicitation of the CS responses by local Ag challenge and is called CS-initiation. This process is marked by a 2-h ear swelling and overall is required to recruit the CS-effector T cells to mediate classical 24 h CS [5]. B-1 cells in the peritoneal cavity become activated within only 1 h after contact sensitization. It is suggested that some hapten conjugates dispersed from the skin site of sensitization [7] are rapidly drained to the peritoneal cavity where B-1 cell surface IgM receptors specific for the hapten cause stimulation of the B-1 cells. However, it is postulated that such antigen stimulation is necessary but not sufficient for full activation of the B-1 cells for their subsequent participation in CS. In addition, IL-4 is required.

NK T cell IL-4 activates the B-1 cells

The new preliminary results suggesting involvement of NK T cells are that CS responses are defective in mice that are deficient in NK T cells, via either deletion of Vα14 Jα281 or CD1d [3]. The defect of CS in NK T cell deficient mice results in partial but significantly impaired 24 h CS responses, and complete absence of the 2 h CS-initiating responses that are due to the B-1 cells. It was shown that CS-effector T cells are intact, and thus it was postulated that activation of CS-initiating B-1 cells is defective in NK T cell deficient mice, abrogating local recruitment of the CS-effector T cells. In fact, there indeed is defective CS-initiation in NK T cell deficient mice. Moreover, deficient CS can be reconstituted by injections of IL-4 at immunization, that normally is derived from early activated NK T cells [3]. The target of IL-4 appears to be the B-1 cell. Thus, CS in NK T cell deficient mice also can be reconstituted by transfer of immunized B-1 cells taken from sensitized wild type mice where NK T cell-derived IL-4 was available [3]. Therefore, we postulate that NK T cell released IL-4, and the TNP-antigens dispersed from the skin site of contact sensitization, both are needed to activate the B-1 cells. This results in migration of B-1 cells to the spleen and lymph nodes, likely to differentiate into plasma cells to produce specific antihapten IgM antibody within one day [5,11–13]. This formulation is similar to the explanation about findings of peritoneal B-1 cells migrating to the mesenteric lymph nodes of the GI tract to produce IgA antibodies [14]. In CS, this B-1 cell derived specific circulating IgM antibody plays a crucial role in the elicitation of the final CS response by enabling the local recruitment of the CS-effector $\alpha\beta$-T cells into the tissues at the site of Ag challenge (unpublished observation).

CS-assisting $\gamma\delta$-T cells

The third-acting lymphocyte subset involved in CS are $\gamma\delta$-T cells that play a crucial role by assisting the CS-effector $\alpha\beta$ T cells. The $\gamma\delta$ T cell-mediated assistance may be due to their $\gamma\delta$ TCR recognizing activation markers in the tissues, perhaps on the $\alpha\beta$ T cells, in order to optimize their responsiveness to the APC. The enigmatic CS-assisting role of the $\gamma\delta$-T cells will be discussed later in this review.

INTERIM SUMMARY

Four specific cells are involved in CS:

- CS-inducing NK $\alpha\beta$-T cells probably activated by dispersed hydrophobic ligands generated during contact sensitization;
- CS-initiating B-1 cells in the peritoneal cavity that probably are coactivated by dispersed specific hapten antigen complexes, together with IL-4 from the NK T cells, to produce IgM to mediate CS-initiation;
- the recruited CS-effector $\alpha\beta$-T cells activated via hapten-self peptide MHC complexes on Langerhans dendritic cells;
- CS-assisting $\gamma\delta$-T cells probably arizing in the spleen and activated at immunization to enter the circulation [15], and probably also are recruited locally to aid the CS-effector $\alpha\beta$-T cells.

The final elicitation of inflammatory responses by the CS-effector $\alpha\beta$-T cells in the skin following local secondary Ag challenge, thus depends on the cooperative activities of the three additional cells. Without the NK T, B-1, or $\gamma\delta$-T cells, despite the presence of the $\alpha\beta$-T cells, no CS is elicited (Fig. 1).

Required CS-initiation involves all four cells

At the time of secondary antigen challenge at a new skin site to elicit CS, local hapten self antigen complexes bind the IgM antibody derived from the circulation (unpublished observation), that was produced earlier by distant B-1 cells likely coactivated by dispersed TNP-Ag and by NK T cell-derived IL-4 [3]. The IgM-Ag complex activates complement causing local elaboration of C5a anaphylatoxin. Our results show that C5a is present in CS ear extracts within the first hour following Ag elicitation of CS [16]. The C5a binds to C5a receptors; perhaps on endothelium, but most importantly on local mast cells and platelets [17]. This causes the mast cells to release vasoactive TNF-α [16–18] and serotonin [19], and also serotonin from platelets [17]. These vasoactive mediators stimulate the local post capillary venules, inducing expression of adhesion molecules like ICAM-1 and VCAM-1 on the luminal surface [18]. Expression of adhesion molecules on the local vasculature enables passing, recently activated, recirculating CS-effector $\alpha\beta$-T cells to adhere via expressed integrins, and then be recruited locally into the tissues. Among these traversing activated T cells, a few have $\alpha\beta$-TCR specific for the hapten-peptide-MHC-complexes on the local APC, that were derived from the secondary hapten Ag challenge. Very few recruited Ag-specific effector T cells [20] likely need to be activated to produce Th1 cytokines like IFN-γ (16) that orchestrate the local inflammatory response. Inflammation is produced by IFN-γ via induction of local tissue cells like keratinocytes to produce chemokines, such as IP-10 [16], MIG, and I-Tac that act on CXCR3 leucocyte chemokine receptors. This attracts and activates nonspecific bone marrow-derived leucocytes to migrate into the site to constitute the perivascular infiltrate of subsequent

tissue inflammation and swelling that characterizes these responses. We postulate that the CS-assisting $\gamma\delta$ T cells are also recruited by CS-initiating mechanisms to optimize the effector $\alpha\beta$-T cell responses. Indeed, it has been shown that $\gamma\delta$-T cells are a major component of the cellular infiltrate in CS responses of lambs, that like other ruminants have large numbers of circulating and lymphoid $\gamma\delta$-T cells [21].

Others have suggested that processes akin to CS-initiation may be due to contact hapten induced direct skin activation; primarily stimulation of keratinocytes to release cytokines via nonantigen-specific activation of NF-κB proinflammatory gene transcription pathways [22]. Although this may apply to some situations, our data show that at sites of CS elicitation, local generation of C5a is Ag-specific [16]. Further, despite the appropriate specific hapten challenge that undoubtedly activates keratinocytes at elicitation, the presence of circulating sensitized T cells alone does not permit their local recruitment to elicit the CS responses [2,11–13, unpublished observation]. In fact, Ag-specific CS-initiation is required [unpublished observation,13].

Possible CS-initiation in clinical responses

Our unpublished results also indicate that related DTH responses to secondary challenge with soluble protein antigen also feature a B-1 cell mediated initiating cascade that similarly recruits DTH-effector T cells into the tissues. Therefore, the CS/DTH-initiating process may be a general phenomena and central to *in vivo* acquired T cell immunity in many instances. Thus, analogous DTH-initiation recruitment into the tissues of various effector T cell subsets, such as Th1 [16], Th2 [23] and Tc1 [24], may lead to local cytokine mediated inflammatory mechanisms that are the basis of microbial resistance, tumour immunity, organ specific autoimmunity and some allergies; including aspects of asthma and atopic dermatitis.

From a clinical perspective, our findings offer a new pathway for triggering these delayed hypersensitivity and related reactions, and hence potentially provide new routes for therapeutic intervention. B cells are already known to participate in a variety of T cell mediated disease models of mice, including auto-immunity in collagen arthritis [25], NOD diabetes [26], lupus-like lesions of lpr/lpr mice [27], encephalomyelitis [28], and T cell protection against infections [29,30]. To date the participation of B cells in these diseases has largely been interpreted as due to participation in afferent APC function [31]. However, our findings, in contrast, suggest that antibodies may participate in elicitation of the effector T cell responses in these diseases, particularly in models like collagen arthritis and encephalomyelitis, where B cells [25,28], antibodies [32,33], complement [34,35] and mast cells [37,38] have been implicated in the efferent T cell responses.

For CS and DTH responses elicited soon after immunization such as on day 4, early activated antigen-specific B-1 cells produce the initiating IgM, and by day 4 Ag-MHC-specific effector T cells become sensitized to mediate the subsequent effector limb that follows antigen challenge to elicit CS reactions in the skin. In responses occurring later, B-1 cells fade (unpublished observation), and B-2 cell-produced isotypes become responsible (unpublished observation) likely complement activating IgG_2 antibodies. Even IgE and IgG_1 antibodies, that in mice mediate T cell recruitment by directly activating mast cell release of vasoactive mediators, are able to mediate CS-initiation in a complement-independent process [25,38].

The CS-assisting $\gamma\delta$ T cells

Yokozeki *et al*. [4] now confirm the important role of the CS-assisting $\gamma\delta$-T cell subset. They studied a novel CS system to the contact antigen para-phenylenediamine, which is widely used in hair dyes and is responsible for clinical contact dermatitis. BALB/c mice immunized multiply by topical painting with 2·5% solution and ear challenged on day 5, elicited small but significant 2 h CS-initiating responses, and larger 24 h CS-effector responses in a hapten-specific manner. Adoptive cell transfers suggested an early acting activated B cell population ($B220^+$, Thy-1^+, $CD5^+$, $\alpha\beta^-$, $\gamma\delta^-$, $CD3^-$, $CD4^-$, $CD8^-$), identical in phenotype to previously described CS-initiating B-1 cells [39–41] was responsible for the early responses, and $CD4^+$ CS-effector $\alpha\beta$-T cells mediate the 24 h response; importantly with the aid of CS-assisting $\gamma\delta$-T cells [4]. As in PCl CS [2], depletion of the $\gamma\delta$-cells substantially diminished transferred CS responses mediated by the CS-effector $\alpha\beta$-T cells [4]. Also, as before [2], transferred CS was reconstituted by adding back the CS-assisting $\gamma\delta$ T cells to the immune CS-effector $\alpha\beta$-T cells [4]. Again [2], the CS-assisting $\gamma\delta$-T cells were neither Ag-specific nor MHC restricted, and also $\gamma\delta$-T cells from normal spleen could assist isolated CS-effector $\alpha\beta$-T cells in adoptive transfer of CS [4,15]. RT-PCR determined the cytokine mRNA pattern in the CS-effector $\alpha\beta$ *versus* CS-assisting $\gamma\delta$-T cells. The $\alpha\beta$-T cells strongly expressed IFN-γ as expected, while the $\gamma\delta$-T cells express not only IFN-γ but also IL-4 and IL-10 [4], suggesting a potential role for these cytokines in CS-assistance.

Possible mechanisms of CS assistance by $\gamma\delta$ T cells

The mechanism of $\gamma\delta$ T cell participation in CS remains obscure. There are at least three possibilities. Firstly, we postulate that some CS-assisting $\gamma\delta$ T cells mobilized at immunization from the spleen into the circulation [15], are recruited locally at the site of challenge by the B-1 cell mediated CS-initiating process. The presence of CS-assisting $\gamma\delta$-T cells in normal spleen shows they are present before immunization. Their absence in nude or SCID mice shows they require thymic differentiation and rearrangement of TCR receptor genes. Since the $\gamma\delta$-T cells are neither Ag-specific nor MHC restricted [2,4], it is postulated that $\gamma\delta$ -T cells assist by recognizing surface expressed self activation antigens on the co-recruited CS-effector $\alpha\beta$-T cells, or on other cells such as APC or local tissue cells. Alternatively, or in addition, $\gamma\delta$-TCR recognition of activation antigens may induce the CS-assisting $\gamma\delta$-T cells to produce cytokines like IL-4 or IL-10 that somehow optimize the CS-effector $\alpha\beta$-T cell responses.

Secondly, the $\gamma\delta$-T cells may have a 'contrasuppressive' regulatory function by making the CS-effector T cells resistant to endogenous suppressor cells that maintain homeostasis in the normal recipients. Different pretreatments of recipients of transfers with the isolated CS-effector $\alpha\beta$-T cells (the $\gamma\delta$-T cells are removed) give insight into the mechanism of action of the CS-assisting $\gamma\delta$ T cells. Recipients treated with *Bordetella pertussis* [15] do not need exogenous $\gamma\delta$ T cells to express CS, since normal splenic $\gamma\delta$ T cells became activated, and are mobilized into the circulation by the *Bordetella* injections, to then serve the CS-assisting function. Also, treatment of recipients with a low dose of cyclophosphamide thought to interfere with suppressor cells, or with mAb against determinants on suppressive cells, also restored transfers by CS-effector $\alpha\beta$-T cells in normal mice again without adding CS-assisting $\gamma\delta$ T cells [15]. This suggested that suppressor cells normally present in the recipients ordinarily

antagonize transfers mediated by CS-effector $\alpha\beta$-T cells, and that the CS-assisting $\gamma\delta$-T cells may serve a protective or contrasuppressive function to block this endogenous suppression, perhaps by rendering the $\alpha\beta$-T cells resistant to suppression.

A third possibility is that the $\gamma\delta$ T cells, like the B-1 cells are activated by IL-4 or other cytokines [42], that perhaps also are derived from early glycolipid activation of the NK T cells [3,5,6], to help mobilize the $\gamma\delta$-T cells from their normal inactive site in the spleen to migrate into the circulation to be able to act as CS-assisting cells after recruitment to the local Ag elicitation site via CS-initiation. Note that these three formulations are not mutually exclusive. Further, a 1-day immune population described previously [43], has a mixed phenotype, that may represent a mixture of B-1 cells and NK T cells that together allowed cultured lines of mixed $\alpha\beta$ and $\gamma\delta$ T cells, that alone only transfer CS locally, to be able to systemically transfer CS [42–44,unpublished observations]; thereby also accounting for the four lymphocyte subsets that mediate CS. In these studies, the artificial procedure of local transfer of CS-effector $\alpha\beta$-T cell lines, which skips over the CS-initiation requirement, may also obviate the need for CS-assistance since so many effector T cells are transferred. This is compared to the very few Ag-specific CS-effector $\alpha\beta$-T cells that likely are recruited naturally [2], and thus their small numbers may require assistance in actively sensitized mice, or in recipients of systemic cell transfers.

Finally, the CS-assisting $\gamma\delta$-T cells preferentially use restricted variable region gene segments [44–46]. This preferential and unusual Vγ and Vδ-TCR gene expression leads to the hypothesis that particular host expressed antigens, such as activation markers or heat shock proteins, likely on the surface of the activated $\alpha\beta$-T cells (or APC, etc.), might serve as ligands for the $\gamma\delta$-TCR on the CS-assisting T cells. Thus, we suggest that CS-assisting $\gamma\delta$-T cells are recruited locally with the CS-effector $\alpha\beta$-T cells via the CS-initiation process that depends on NK T cell-derived IL-4, B-1 cell-derived IgM, and mast cell-derived TNF-α and serotonin. Then in the extravascular tissues, while the CS-effector $\alpha\beta$-T cells are interacting via cognate specificity with hapten-peptide-MHC complexes on APC, the $\gamma\delta$-TCR of CS-assisting cells interact via their $\gamma\delta$-TCR with either native activation proteins, or with low molecular weight phenyl pyrophosphate or alkamine-like ligands, expressed via the activated $\alpha\beta$-T cells, or APC or keratinocytes, etc., to then assist the $\alpha\beta$-T cells. The surface expressed antigens that activate the $\gamma\delta$-TCR could be heat shock proteins or minor histocompatability antigens that become newly expressed via nonspecific skin cell activation via NF-κB pathways that are stimulated by the irritating nonspecific contact hapten challenge [21]. Binding of $\gamma\delta$-TCR to these surface complexes may then activate the $\gamma\delta$-T cells to positively influence the recruited CS-effector $\alpha\beta$-T cells, thereby optimizing their production of Th1 cytokines to subsequently generate full elicitation of CS inflammation.

CONCLUSION

We have summarized recent data showing that *in vivo* T cell mediated immunity in contact sensitivity involves four specific lymphocyte subsets, including three types of T cells and also B-1 cells. Therefore, the required involvement of several previously unanticipated processes that take part in interactions between these lymphocyte subsets, raises the possibility that immune resistance, or deleterious allergic and autoimmune processes that are mediated by these mechanisms, may be susceptible to new treatment modalities that are based on this knowledge.

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