Immunology 1995 **84** 105–110

Interaction between γδ T cells and B cells regulating IgG production

G. HÄCKER,* S. ADAM & H. WAGNER *Institute of Medical Microbiology and Hygiene, Technical University of Munich, Munich, Germany*

SUMMARY

Despite profound knowledge about the molecular structure of the γδ T-cell receptor (TCR), the physiological function of γδ T cells remains enigmatic. Participation of these cells in complex immune reactions, however, is suggested by the appearance of γδ T cells in sites of infectious and autoimmune-induced inflammations. Only a few *in vitro* models of γδ T-cell stimulation have been established: besides a reactivity in the presence of microbial ligands, human γδ T cells proliferate upon *in vitro* challenge with cells from an allogeneic B-lymphoblastic cell line (B-LCL). We present data here demonstrating that this reactivity is not confined to allogeneic B-LCL. Autologous B-LCL are also very strong stimulators for γδ T cells; more important, autologous B cells can stimulate γδ T cells after a period of mitogen-activation but not in a resting state. This activation seems to address a subgroup of γδ T cells, as the percentage of Vδ1$^+$ cells is increased after stimulation. Activated γδ T cells, on the other hand, are able to exert an influence on B cells by inhibiting the secretion of IgG in coculture experiments. These data define a simple regulatory circle of B cells and γδ T cells *in vitro* and propose a model for γδ T-cell function which could explain many *in vivo* observations of γδ T-cell activation.

INTRODUCTION

γδ T cells are a population of CD3$^+$ cells in lymphoid tissues and epithelia,[1,2] comprising about 5% of T cells in human peripheral blood.[3] Although the molecular structure of the γδ T-cell receptor (TCR) is well known and is similar to the αβ TCR, the functions and the physiological role of these cells remain ambiguous. Previous work has shown that γδ T cells become activated in cultures containing various microbial ligands, e.g. mycobacteria,[3,4] streptococci[5] and plasmodia,[6] but neither the molecular structure of these ligands nor a pattern of ligand presentation has been worked out until now. On the other hand, there is much evidence of the participation of γδ T cells in complex *in vivo* reactions: many studies have demonstrated that the defence against very different invading pathogens, ranging from bacteria[7] and viruses[8] to worms,[9] seems to include an activation of γδ T cells in natural as well as in experimental infections. It should be noted, however, that in most cases no specificity against the infectious agent has been demonstrated.

In humans, the peripheral blood γδ T-cell subset is mainly comprised of two groups with different TCR V-segment expression. These two subgroups are defined by either the Vδ2 TCR segment, paired in most cases with the V9 segment of the TCR γ-chain, or the Vδ1 segment together with one of various Vγ proteins.[3,10] Whereas the microbially induced reactivity is confined to the Vγ9δ2 subset,[3,4,11] we have recently described an *in vitro* reactivity of γδ T cells from peripheral blood carried mainly by the Vδ1$^+$ T cells.[10] This reactivity consists of both a strong proliferative response upon challenge with cells from allogeneic Burkitt's lymphoma (BL) cell lines or allogeneic Epstein–Barr virus (EBV)-transformed B cell lines (B-LCL), and a marked shift to the Vδ1$^+$ subgroup in the responding population. In investigating this system further, we addressed two questions. First, does the reactivity of this γδ T-cell population extend on autologous B-LCL cells? Second, is it possible to induce this γδ T-cell-stimulating ligand on normal resting B cells, giving them stimulatory potential towards γδ T cells? Here we show data demonstrating that in fact γδ T cells can be stimulated by autologous B cells after EBV transformation or after a period of mitogen activation. Furthermore, γδ T cells activated by these B cells are in turn able to exert an influence on B-cell function by actively suppressing the secretion of IgG.

Received 17 May 1994; revised 5 August 1994; accepted 31 August 1994.

Abbreviations: BL, Burkitt's lymphoma; B-LCL, B-lymphoblastic cell line.

*Current address and correspondence: The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Victoria 3050, Australia.

MATERIALS AND METHODS

Preparation of a purified γδ T-cell population

Purification of γδ T cells was performed as described previously.[4] Briefly, mononuclear cells (MNC) of healthy volunteers were isolated by Ficoll–Hypaque (Pharmacia, Freiburg, Germany) density gradient centrifugation. MNC were E-rosetted with neuraminidase-treated sheep red blood

cells. Depletion of αβ T cells and natural killer (NK) cells from E-positive cells was carried out by, first, labelling cells with BMA031 (anti-αβ TCR; a generous gift of Dr R. Kurrle, Behringwerke, Marburg, Germany) and Leu-11b (anti-CD16, NK cells; Becton Dickinson, Heidelberg, Germany) monoclonal antibodies (mAb), respectively. In a second step, biotinylated goat anti-mouse IgG $F(ab')_2$ (Tago, Burlingame, CA), and in a third step FITC-conjugated avidin (Dianova, Hamburg, Germany), was added. Afterwards, cells were incubated for 5 min on ice with biotinylated iron microbeads and run over a magnet-activated cell sorter (MACS) column (Stefan Miltenyi Biotech, Bergisch Gladbach, Germany). Cells not retained in the column were stained with phycoerythrin-coupled Leu-19 (anti-CD56; Becton Dickinson, recognizing NK subset and activated T cells), and cells staining positive either for FITC (cells remaining after MACS purification) or phycoerythrin were sorted out by FACS (Epics Elite, Coulter Electronics, Hialeah, FL). The purity of γδ T cells was checked by staining and analysing in FACS either immediately after the purification procedure or after 7 days culture with phytohaemagglutinin (PHA) and interleukin-2 (IL-2); in all experiments reported here, contamination by αβ T cells was below 10%.

Phenotype analysis

T-cell populations were stained with mAb TCRγδ1–FITC (recognizing all γδ T cells), δTCS1 (specific for the Vδ1 segment; both T Cell Sciences, Cambridge, MA) or 4G6–FITC (Vδ2 specific)[4] in combination with Leu-4 (anti-CD3)–phycoerythrin (Becton Dickinson). Per sample, 5000 viable cells were analysed using an Epics Elite flow cytometer (Coulter).

Stimulator cells

B-LCL were generated by infecting an E-negative population of MNC with EBV strain B95–8 secreted by the marmoset cell line B95–8 (ATCC, Rockville, MD). Transformed cells were cultured in RPMI-1640 (Biochrom, Berlin, Germany) supplemented with antibiotics and 5% fetal calf serum (FCS; Biochrom), and were used in stimulation experiments after a period of at least 4 weeks following EBV infection.

Mitogen-stimulation of cells from peripheral blood was performed using pokeweed mitogen (PWM; Gibco, Eggenstein, Germany) or formalin-treated *Staphylococcus aureus* Cowan strain (SAC; Sigma Chemicals, Munich, Germany). Populations stimulated were whole MNC, E-positive cells, E-negative cells or E-negative cells depleted of macrophages by incubating cells in a 0·05 M solution of L-leucine methyl ester (L-LME; Sigma) in phosphate-buffered saline (PBS) for 35 min at room temperature;[12] the latter population contained about 90% B cells, as determined by staining with CD19-specific mAb (Becton Dickinson). Cells were incubated at a density of 2×10^6 with either PWM (1 : 20 dilution) or SAC (0·01%) in RPMI containing 10% FCS for various times, as indicated in the table legends, washed three times, irradiated (20 Gy) and used as stimulators for γδ T cells.

Proliferation assays

Replicate cultures of 3×10^3 purified γδ T cells were cultured in the presence of 5×10^4 irradiated (20 Gy) autologous feeder cells (MNC). As indicated in the legends, irradiated stimulator cells (20 Gy/10^4 cells/well, or in the case of B-LCL, 40 Gy/5×10^3 cells/well) or PHA (0·5 μg/ml; Wellcome, Heidelberg, Germany) were added. Culture medium was RPMI-1640 containing 20 mM L-glutamine, 10 mM HEPES, antibiotics and 10% heat-inactivated human serum. After 3 days of culture, 10 U/ml of rIL-2 (Eurocetus, Amsterdam, the Netherlands) was added; on day 8 cultures were pulsed with [^{3}H]thymidine (740 KBq/ml) for 4 hr, collected onto filter papers using a 96-well harvester (Inotech, Asbach, Germany) and counted by direct β-counting in a Matrix-96-β-counter (Packard, Frankfurt, Germany). Proliferative responses are given as mean values of four replicate cultures ± SD.

Measurement of IgG in supernatants

B-cell preparations (1×10^5/well) were stimulated in flat-bottomed microtitre plates with SAC (0·01%), either alone or in the presence of cells from αβ or γδ T-cell lines (5×10^4/well), in triplicate for 5 or 6 days. After this period, supernatants were assayed for IgG content in an ELISA using standard protocols. Goat anti-human IgG and Fc-specific peroxidase-coupled goat anti-human IgG (Jackson Laboratories, distributed by Dianova, Hamburg, Germany; cross-absorbed against serum proteins from other species) were used as specific antibodies. Standard IgG preparations were from Sigma Chemicals. 3,3′,5,5′-tetramethylbenzidine dihydrochloride (Sigma) was used as substrate, and extinction was measured in an ELISA reader at 450 nm.

RESULTS

The capability of human Vγ9δ2$^+$ T cells to react against seemingly unrelated microbial ligands has been known for several years.[3,13] Recently, we have described a new model of reactivity of human γδ T cells *in vitro*:[10] primary purified γδ T cells can be stimulated to proliferate *in vitro* by cells from BL and B-LCL. One example of this reactivity is shown in Table 1: besides the proliferative response a shift in the γδ T-cell population towards Vδ1$^+$ at the expense of the Vδ2+ subgroup is seen. Because some individual γδ T-cell clones have been found to lyse target cells in an apparently major histocompatibility complex (MHC)-restricted manner,[14] and because this reaction resembles the strong activation of αβ T cells by

Table 1. Allogeneic B-LCL stimulate primarily Vδ1$^+$ γδ T cells

Stimulus	Percentage of cells positive for			Proliferation (SD)
	γδ TCR	Vδ1	Vδ2	
None		ND		35 (21)
PHA	ND	8·1	86·6	26 604 (5307)
B-LCL allogeneic	94·7	60·5	16·3	8622 (3270)

Purified γδ T cells (2×10^3/well) were cultured in microtitre plates in the presence of autologous irradiated MNC as feeder cells (5×10^4) without stimulus, with PHA (0·5 μg/ml) or with irradiated cells from an allogeneic B-LCL (5×10^3). Recombinant IL-2 was added on day 3 of culture, and proliferation of six replicate cultures and Vδ distribution by staining a pool from six replicate cultures were determined on day 7, as described in the Materials and Methods. Data give the means and the SD of six replicate cultures. ND, not done.

Table 2. B-LCL stimulate proliferation of autologous γδ T cells

Stimulator cells	Proliferative response (c.p.m./SD)		
	Donor 1	Donor 2	Donor 3
No cells	78 (90)	129 (118)	669 (786)
B-LCL allogeneic	3913 (2223)	7032 (2907)	8310 (1431)
B-LCL autologous	3894 (2934)	3490 (1357)	3936 (2756)

B-LCL were established by transforming B cells from peripheral blood of three healthy donors. At least 4 weeks later, γδ T cells were purified from a second blood sample of the same donors and cultured in microtitre plates (3×10^3/well) in the presence of irradiated autologous feeder cells (5×10^4) and either cells from the autologous or from an arbitrarily chosen allogeneic B-LCL (5×10^3/well). Recombinant IL-2 was added on day 3, and proliferation was measured on day 7 of culture.

allogeneic B-LCL, the question had to be resolved whether this kind of reactivity in γδ T cells against allogeneic B-LCL is the expression of an alloreactivity, i.e. a recognition of foreign MHC molecules, or a stimulation by a ligand on the B-LCL related to their state of transformation. To distinguish between these two possibilities, we performed the same stimulation assay in an autologous system: two blood samples were drawn from the same donor at different times with an interval of 4–6 weeks. E-rosette-depleted cells from the first sample were infected with EBV strain B95–8 to generate B-LCL, and γδ T cells purified from the second sample were stimulated with cells from these B-LCL. As depicted in Table 2, in all three donors tested we found a strong proliferative response of a purified γδ T-cell population to autologous B-LCL, which was comparable to the stimulation by allogeneic B-LCL cells; supernatants from B-LCL had no significant effect (data not shown). Because resting B cells are not stimulatory for γδ T cells (Table 5), it is evident that by the process of EBV-infection and transformation a ligand was induced on B cells capable of stimulating autologous γδ T cells.

Table 3. Mitogen-activated B cells stimulate autologous γδ T cells

Stimulus	Percentage of cells positive for			
	γδ TCR	Vδ1	Vδ2	Proliferation
None		ND		220 (166)
PHA	98·0	16·6	77·0	21 289 (1559)
PWM-stimulated cells	92·9	65·8	31·9	4146 (1323)
SAC-stimulated cells	97·3	42·7	32·0	4888 (1061)

MNC from a healthy donor were stimulated for 2 days with either PWM (1 : 20 dilution) or SAC (0·01%) at 2×10^6 cells/ml; these cells are referred to as 'PWM-simulated cells' and 'SAC-stimulated cells', respectively. After these 2 days, γδ T cells from the same donor were purified from a fresh blood sample and stimulated with either PHA (0·5 μg/ml; 2×10^3 γδ T cells/well) or irradiated PWM- or SAC-activated cells (10^4 cells/well) in the presence of autologous feeder cells (5×10^4/well). IL-2 was added on day 3, and proliferation and Vδ distribution in the responding γδ T-cell population (each in six replicate cultures) were determined. Proliferation is given as c.p.m. (SD).

EBV transformation led to a very strong activation of the infected B cells. Therefore, we asked whether an activation of B cells *in vitro* with mitogen would yield the same effect. Two potent mitogens are known for human B cells: PWM stimulates B cells only in the presence of T cells, whereas SAC can act as a mitogen on B cells independent of T cells. Table 3 shows the results of this experimental approach: MNC from a healthy donor were isolated and stimulated with PWM or with SAC. Two days later, γδ T cells were isolated from the same donor, stimulated with these mitogen-activated and irradiated MNC, and after 7 days of culture proliferation and Vδ subset distribution were measured. As suspected, a clear proliferative response of the purified γδ T-cell population could be seen; this reactivity followed the same rules as the reaction against B-LCL, i.e. a prominent shift towards the $V\delta1^+$ γδ T-cell subset (Table 3). The stimulation with allogeneic SAC-stimulated MNC gave very similar results (data not shown).

In order to demonstrate that this effect was due to the activation of B cells, we followed the stimulus through separate cell populations. Starting from autologous MNC, this population was divided first by rosetting with sheep erythrocytes in an E-rosette positive (T cells and NK cells) and an E-rosette negative (predominantly B cells and macrophages) population, and then by further treatment of the E-negative population with L-LME, which selectively destroys macrophages, a population containing about 80–90% B cells was prepared. Table 4 details results of experiments with three individual donors and demonstrates that the stimulatory activity was by far the strongest in the population of purified B cells. The proliferation correlated with the number of stimulator cells (data not shown), and the stimulatory effect was much stronger after a 16-hr SAC incubation of the B cells at 37° compared to a 30-min incubation at 4° (Table 5), indicating that an active process in the B cells was necessary to induce the stimulating capability towards γδ T cells.

This effect of an activation-induced γδ T-cell ligand on B cells led us to question the physiological impact of this kind of interaction. If activated B cells can stimulate γδ T cells, we expected the activated γδ T cells to have an influence on the function of B cells. The physiological possibilities of this influence include two principal options: γδ T cells could either support B cells, i.e. express B-cell help, or they could regulate B cells negatively and inhibit their function. To investigate for such an effect we established a set of long-term γδ T-cell lines. Primary isolated γδ T cells were stimulated with SAC-activated MNC, either polyclonally or in limiting dilution cultures, to receive clones. Responding cells were expanded by biweekly stimulation with PHA and continuous supply with IL-2. As a principal B-cell function, we chose the production of IgG: B cells were prepared from peripheral blood and stimulated with SAC to secrete immunoglobulins into the supernatant. Cells from the γδ T-cell lines or, as a control, from long-term αβ T-cell lines kept in culture the same way as the γδ T cells, were added to the (allogeneic) B cells. After 5 or 6 days of stimulation, supernatants were collected and the content of IgG was determined by ELISA. Figure 1 details the results of two experiments: the presence of γδ T cells significantly diminished the production of IgG in B-cell cultures. This was not an unspecific effect of the culture conditions, because the presence of αβ T cells had no significant suppressing influence on the IgG production (Fig. 1). The increase in IgG

Table 4. The stimulatory effect maps to B cells

Exp.	Stimulus/stimulator cell population					
	None	PHA	MNC	E^+	E^-	E^-/L-LME
1	44 (26)	8762 (684)	1243 (1153)	652 (554)	2739 (1050)	ND
2	911 (304)	ND	ND	ND	1340 (246)	5099 (145)
3	468 (525)	7178 (4696)	ND	ND	1952 (959)	5036 (1096)

MNC from peripheral blood of three different donors were divided into separate cell populations, which were incubated with SAC (0·01%, 2×10^6 cells/ml) in the incubator for 20 hr. During this time, $\gamma\delta$ T cells were purified from the same blood sample and cultured with the washed SAC-stimulated cells in microtitre plates (3×10^3 $\gamma\delta$ T cells, 5×10^4 irradiated feeder cells/well and PHA 0·5 μg/ml or irradiated stimulator cells at 1×10^4 in Exp.1, 4×10^4 in Exp. 2 and 2×10^4 in Exp. 3).

Stimulator cell populations used were whole MNC, E-rosette positive (E^+) or E-rosette-depleted (E^-) MNC or a population of E^- cells depleted of macrophages by treatment with E^-/L-LME; this population contained about 80–90% B cells. Recombinant IL-2 was added on day 3 and proliferation was measured on day 7. Proliferation is given as c.p.m. (mean of six replicate cultures) (SD).

concentration in some cultures containing $\alpha\beta$ T cells was probably due to the production of lymphokines supporting B-cell function by the T cells. A restriction to any MHC molecules seems unlikely; $\gamma\delta$ T cells from different donors, stimulated with autologous activated B cells, were able to exert a suppressing effect on the B cells of various arbitrarily chosen donors (data not shown). These observations of interactions between B cells and $\gamma\delta$ T cells point to a regulatory role of this T-cell subset in the immune system.

DISCUSSION

Our data demonstrate two steps of an interaction between $\gamma\delta$ T cells and B cells which fulfil a complete circle of regulation. The activation of B cells renders them targets for $\gamma\delta$ T cells and this recognition by $\gamma\delta$ T cells leads to a deactivation of the B cells. These observations propose a model of function of $\gamma\delta$ T cells embedded in the network of other cells of the immune system and acting as negative regulators of the immune response.

Table 5. A short-term incubation with SAC is not sufficient for $\gamma\delta$ T-cell stimulation

Exp.	Stimulus/ SAC-incubation of stimulator cells				
	None	PHA	No SAC	SAC 30 min/ice	SAC 16 hr 37°
1	44 (26)	8762 (684)	ND	779 (780)	2739 (1050)
2	305 (315)	3571 (1299)	663 (351)	579 (275)	1213 (371)
3	79 (26)	6438 (741)	442 (354)	971 (637)	2219 (759)

$\gamma\delta$ T cells were stimulated with autologous cells (Exp. 1: E^- population, same experiment as Exp. 1 in Table 4; Exps 2 and 3: E^-/L-LME, see legend to Table 4), that had been incubated with SAC (0·01%, 2×10^6 cells/well) for either 30 min on ice or for 16 hr at 37° or which were left untreated ('No SAC'). Cultures in microtitre plates contained 3×10^3 $\gamma\delta$ T cells, 1×10^4 irradiated stimulator cells or PHA (0·5 μg/ml) and 5×10^4 irradiated autologous feeder cells. Recombinant IL-2 was added on day 3, and proliferation was measured on day 7 of culture. Values (c.p.m.) give the mean and the SD of six replicate cultures.

$\gamma\delta$ T cells have been implicated to play roles in various parts of the immune system. The role that has been attributed to them most often is a function of a first line of defence against invading pathogens.[15,16] This idea is deduced from two findings: first, from the distribution of $\gamma\delta$ T cells throughout epithelial tissues in some species, as in the mouse,[17] although this is not true for other species such as humans;[17,18] second, from the observations of responses of $\gamma\delta$ T cells in the context of microbial infections *in vivo*[7,19] and upon *in vitro* challenges with microbial ligands.[3,6] Others have suspected a regulatory role for $\gamma\delta$ T cells:[20,21] the bases for these models have been reports of a 'self-recognition' of murine $\gamma\delta$ T-cell hybridomas,[22] the response of $\gamma\delta$ T cells from murine skin towards stressed keratinocytes,[23] and the capability of $\gamma\delta$ T cells to produce a wide panel of lymphokines[24,25] which gives these cells the chance to influence other cell populations. Our model of a regulatory system is consistent with all of these ideas. According to this model, the observed $\gamma\delta$ T-cell reactivity in complex immune reactions *in vivo*, as in infections[7,19] or in cases of autoimmune diseases,[26–28] is not directed against the pathogen itself but against B cells and perhaps other components of the immune system responding to the invading microbes or to autoantigens. The *in vivo* situation, which most closely resembles our *in vitro* system, is the cell composition in human immunodeficiency virus (HIV)-infected individuals. These patients harbour in the peripheral blood increased numbers of activated B cells[29,30] and their $\gamma\delta$ T-cell pool is shifted to the $V\delta1^+$ subgroup; these $V\delta1^+$ cells express the HLA-DR molecule as an activation marker on their surface.[31,32]

Several investigators have reported evidence for an interaction of $\gamma\delta$ T cells and other lymphocyte subpopulations both *in vivo* and *in vitro* and, depending on the situation, either a positive or a negative effect of $\gamma\delta$ T cells on other cells has been found. Like $CD4^+$ $\alpha\beta$ T cells, the very small subgroup of $CD4^+$ $\gamma\delta$ T cells, but not $CD4^-$ $CD8^-$ $\gamma\delta$ T cells, has been reported to enhance the IL-2-driven secretion of immunoglobulins by splenic B cells in a TCR-independent fashion.[33] In mice, a $CD4^-$ $CD8^-$ $\gamma\delta^+$ lymphocyte population in the lymph node could induce the differentiation of a syngeneic B-cell lymphoma.[34] Some examples of negative actions by $\gamma\delta$ T cells on other cells have been also reported. A suppressive effect has been described in a system of

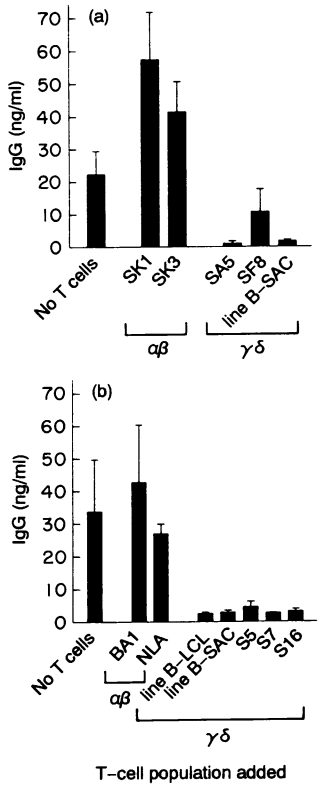

Figure 1. γδ T cells suppress IgG production. B cells were purified from peripheral blood as detailed in the Materials and Methods. B-cell populations were stimulated in flat-bottomed microtitre plates at 1×10^5/well, with a 0·01% SAC suspension, in triplicate either alone or in the presence of the indicated long-term T-cell line. On day 6 supernatants were assayed for IgG and IgM content. The following T-cell lines were used: BA1 and NLA (polyclonal alloreactive αβ T-cell lines, raised against third-party alloantigens and not reactive against the B cells used in this experiment), polyclonal γδ T-cell lines activated by allogeneic B-LCL (line B-LCL) or autologous SAC-activated B cells (line B-SAC), alloreactive αβ T-cell clones (SK1, SK3) and γδ T-cell clones raised primarily in limiting-dilution cultures by stimulation with SAC-activated autologous E^- MNC (SA5, SF8, S5, S7, S16). (a) and (b) refer to independent experiments using MNC from different donors and a different set of T-cell lines. The Vδ distribution in the cell lines used was approximatedly 60% $V\delta1^+$ and 30% $V\delta2^+$ cells, the γδ T-cell clones used were exclusively of the Vδ1 phenotype.

antibody production by chicken cells, which was alleviated by the removal of γδ T cells;[35] a suppression of IgE production in rats was carried out by a $CD3^+$ $\alpha\beta^-$ cell population;[36] and γδ T cells have been found to be able to inhibit erythropoiesis in a patient with pure red cell aplasia.[37] Data which may be most closely related to our findings are reported in cattle, where the *in vivo* depletion of a $CD2^-$ $CD4^-$ $CD8^-$ population, presumably a homologue of human γδ T cells, led to an enhanced antibody response upon *in vivo* challenge.[38]

The finding not completely congruent with the present model is the exclusive restriction of anti-microbial reactivity of γδ T cells *in vitro* to distinct Vγ/Vδ segments. The reactivity of human γδ T cells against *Mycobacterium tuberculosis*,[3] apathogenic mycobacteria,[39] streptococci[5] and plasmodia[6] seems to involve only $V\gamma9/\delta2^+$ cells; and in mice the response to *M. tuberculosis* is confined to the $V\gamma1/\delta6^+$ γδ T-cell subset.[16] This kind of reactivity, i.e. a restriction to distinct V segments in various species, displays features of superantigen activation in αβ T cells; we therefore believe that this *in vitro* activity is an entity of its own different from a 'normal' activation with a nominal antigen. Interestingly, in a human infection with *M. leprae*, $V\delta1^+$ γδ T cells were found to be also present in infectious granulomas;[19] this could mean that the γδ T-cell reaction does depend on the composition of the reacting immune cells and may be different *in vivo* and *in vitro*.

At the moment we know neither the stimulating ligand nor the mechanism by which γδ T cells switch off B cells. Although a few individual clones have been reported to recognize foreign MHC molecules,[14] and a restriction of γδ T cells on class Ib molecules has been proposed,[40,41] no clear recognition pattern has been worked out. Recent work raises evidence for a new model of MHC recognition by γδ T cells.[42] The authors of that study investigated the antigen recognition in two MHC-restricted γδ T-cell clones and found that the recognition seemed to be specific for empty MHC molecules rather than peptide–MHC complexes. They proposed that the γδ T-cell receptor acts in a more immunoglobulin-like fashion and does recognize antigens without processing and usually not in the context of MHC; the recognition of MHC by a few γδ T-cell clones might therefore be an accident rather than the rule for this cell type. This is in congruence with our findings, that the reactivity of the γδ T cells towards cell-bound antigens does not seem to be determined by the MHC on the stimulator cell.

Regarding the question of the nature of the stimulating ligand, the 65 000 MW heat-shock protein hsp has been pointed out by some authors,[27,43] but this could not be confirmed by others.[3] The view of a stress-induced ligand as target structure, however, is consistent with the model of an activation-dependent recognition of stimulator cells by γδ T cells. In our system a restriction pattern on foreign MHC molecules was not evident, and the stimulation of γδ T cells by B-LCL could not be inhibited by mAb against the MHC class I heavy chain or the β2-microblobulin molecule (data not shown), but we cannot completely exclude a presentation by low-variability structures such as MHC class Ib molecules.

Regarding the effector mechanism of the action of γδ T cells on B cells, two principal possibilities exist, i.e. an effect mediated by secreted lymphokines, or a direct killing of the target cells. At the moment we cannot be sure which one is valid; from present knowledge, γδ T cells should have the principal capabilities to exert either one or both of these functions. This issue has to be resolved by future experimental work.

Taken together, our data describe an interaction between γδ T cells and B cells in both directions, defining a simple regulatory mechanism which could be effective in many *in vivo* situations. Thus these data suggest a physiological role for these enigmatic cells within the network of interactions of the cells of the immune system.

REFERENCES

1. Tonegawa S., Berns A., Bonneville M. *et al.* (1989) Diversity, development, ligands, and probable functions of γδ T cells. *Cold Spring Harb Symp Quant Biol* **54**, 31.
2. Saito H., Kranz D.M., Takagaki Y., Hayday A.C., Eisen H.N. & Tonegawa S. (1984) Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. *Nature* **309**, 757.

3. Kabelitz D., Bender A., Schondelmaier S., Schoel B. & Kaufmann S.H. (1990) A large fraction of human peripheral blood $\gamma\delta^+$ T cells is activated by *Mycobacterium tuberculosis* but not by its 65-kD heat shock protein. *J Exp Med* **171,** 667.
4. Pfeffer K., Schoel B., Gulle H., Kaufmann S.H. & Wagner H. (1990) Primary responses of human T cells to mycobacteria: a frequent set of $\gamma\delta$ T cells is stimulated by protease-resistant ligands. *Eur J Immunol* **20,** 1175.
5. Bender A. & Kabelitz D. (1992) Preferential activation of peripheral blood $V\gamma9^+$ $\gamma\delta$ T cells by group A, B and C but not group D or F streptococci. *Clin Exp Immunol* **89,** 301.
6. Goerlich R., Hacker G., Pfeffer K., Heeg K. & Wagner H. (1991) *Plasmodium falciparum* merozoites primarily stimulate the $V\gamma9$ subset of human $\gamma\delta$ T cells. *Eur J Immunol* **21,** 2613.
7. Emoto M., Danbara H. & Yoshikai Y. (1992) Induction of $\gamma\delta$ T cells in murine salmonellosis by an avirulent but not by a virulent strain of *Salmonella choleraesuis*. *J Exp Med* **176,** 363.
8. Carding S.R., Allan W., Kyes S., Hayday A., Bottomly K. & Doherty P.C. (1990) Late dominance of the inflammatory process in murine influenza by $\gamma\delta^+$ T cells. *J Exp Med* **172,** 1225.
9. McClure S. J., Emery D.L., Wagland B.M. & Jones W.O. (1992) A serial study of rejection of *Trichostrongylus colubriformis* by immune sheep. *Int J Parasitol* **22,** 227.
10. Hacker G., Kromer S., Falk M., Heeg K., Wagner H. & Pfeffer K. (1992) $V\delta1^+$ subset of human $\gamma\delta$ T cells responds to ligands expressed by EBV-infected Burkitt lymphoma cells and transformed B lymphocytes. *J Immunol* **149,** 3984.
11. De Libero G., Casorati G., Giachino C. *et al.* (1991) Selection by two powerful antigens may account for the presence of the major population of human peripheral $\gamma\delta$ T cells. *J Exp Med* **173,** 1311.
12. Splawski J.B. & Lipsky P.E. (1991) Immunologic studies in humans; isolation of B cell populations. In: *Current Protocols in Immunology* (eds J.E.A.M. Kruisbeek, D.H. Margulies, E.M. Shevach & W. Strober Coligan), p. 7.5.1. Greene Publishing Associates and Wiley-Interscience, New York.
13. Kabelitz D., Bender A., Prospero T., Wesselborg S., Janssen O. & Pechhold K. (1991) The primary response of human $\gamma\delta^+$ T cells to *Mycobacterium tuberculosis* is restricted to $V\gamma9$-bearing cells. *J Exp Med* **173,** 1331.
14. Bluestone J.A., Cron R.Q., Cotterman M., Houlden B.A. & Matis L.A. (1988) Structure and specificity of T cell receptor $\gamma\delta$ on major histocompatibility complex antigen-specific $CD3^+$, $CD4^-$, $CD8^-$ T lymphocytes. *J Exp Med* **168,** 1899.
15. Janeway C.A. (1990) $\gamma\delta$ T cells: research on the frontlines of defence. *Res Immunol* **141,** 688.
16. Born W. & O'Brien R. (1990) The $\gamma\delta$ cell response to stress: unresolved issues and possible significance. *Res Immunol* **141,** 595.
17. Borst J., Vroom T.B., Bos J.D. & van Dongen J.J. (1991) Tissue distribution and repertoire selection of human $\gamma\delta$ T cells: comparison with the murine system. *Curr Top Microbiol Immunol* **173,** 41.
18. Vroom T.M., Scholte G., Ossendorp F. & Borst J. (1991) Tissue distribution of human $\gamma\delta$ T cells: no evidence for general epithelial tropism. *J Clin Pathol* **44,** 1012.
19. Uyemura K., Deans R.J., Band H. *et al.* (1991) Evidence for clonal selection of $\gamma\delta$ T cells in response to a human pathogen. *J Exp Med* **174,** 683.
20. Roberts K. & Shevach E.M. (1991) Immunoregulatory role of $\gamma\delta$ T cells. *Ann NY Acad Sci* **636,** 1.
21. Fujihashi K., Taguchi T., Aicher W.K. *et al.* (1992) Immunoregulatory functions for murine intraepithelial lymphocytes: $\gamma\delta$ T cell receptor-positive (TCR^+) T cells abrogate oral tolerance, while $\alpha\beta$ TCR^+ T cells provide B cell help. *J Exp Med* **175,** 695.
22. Roberts K., Yokoyama W.M., Kehn P.J. & Shevach E.M. (1991) The vitronectin receptor serves as an accessory molecule for the activation of a subset of $\gamma\delta$ T cells. *J Exp Med* **173,** 231.
23. Havran W.L., Chien Y.H. & Allison J.P. (1991) Recognition of self antigens by skin-derived T cells with invariant $\gamma\delta$ antigen receptors. *Science* **252,** 1430.
24. Taguchi T., Aicher W.K., Fujihashi K. *et al.* (1991) Novel function for intestinal intraepithelial lymphocytes. Murine $CD3^+$, $\gamma\delta$ TCR^+ T cells produce IFN-γ and IL-5. *J Immunol* **147,** 3736.
25. Spits H. (1991) Human T cell receptor $\gamma\delta^+$ T cells. *Semin Immunol* **3,** 119.
26. Rust C., Pena S., Kluin P. & Koning F. (1990) $\gamma\delta$ T cells in coeliac disease. *Res Immunol* **141,** 668.
27. Holoshitz J., Koning F., Coligan J.F., de Bruyn J. & Strober S. (1989) Isolation of $CD4^-$ $CD8^-$ mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. *Nature* **339,** 226.
28. Holoshitz J. (1990) Potential role of $\gamma\delta$ T cells in autoimmune diseases. *Res Immunol* **141,** 651.
29. Amadori A., Zamarchi R., Ciminale V. *et al.* (1989) HIV-1-specific B cell activation. A major constituent of spontaneous B cell activation during HIV-1 infection. *J Immunol* **143,** 2146.
30. Mizuma H., Litwin S. &. Zolla Pazner S. (1988). B-cell activation in HIV infection: relationship of spontaneous immunoglobulin secretion to various immunological parameters. *Clin Exp Immunol* **71,** 410.
31. Kabelitz D., Pechhold K., Bender A. *et al.* (1991) Activation and activation-driven death of human $\gamma\delta$ T cells. *Immunol Rev* **120,** 71.
32. de Paoli P., Gennari D., Martelli P. *et al.* (1991) A subset of $\gamma\delta$ lymphocytes is increased during HIV-1 infection. *Clin Exp Immunol* **83,** 187.
33. van Vlasselaer P., Cascan H., de Waal Malefyt R. & de Vries J.E. (1992) IL-2 and a contact-mediated signal provided by TCR $\alpha\beta^+$ or TCR $\gamma\delta^+$ $CD4^+$ T cells induce polyclonal IgG production by committed human B cells. *J Immunol* **148,** 1674.
34. Sperling A.I. & Wortis H.W. (1989) $CD4^-$, $CD8^-$ $\gamma\delta$ cells from normal mice respond to a syngeneic B cell lymphoma and can induce its differentiation. *Intern Immunol* **1,** 484.
35. Quere P., Cooper M.D. & Thorbecke G.J. (1990) Characterization of suppressor T cells for antibody production by chicken spleen cells. I. Antigen-induced suppressor cells are $CT8^+$, $TcR1^+$ ($\gamma\delta$) T cells. *Immunology* **71,** 517.
36. McMenamin C., Oliver J., Girn B. *et al.* (1991) Regulation of T-cell sensitization at epithelial surfaces in the respiratory tract: suppression of IgE responses to inhaled antigens by $CD3^+$ TCR $\alpha\beta$ lymphocytes (putative $\gamma\delta$ T cells). *Immunology* **74,** 234.
37. Hara T., Mizuno Y., Nagata M. *et al.* (1990) Human $\gamma\delta$ T-cell receptor-positive cell-mediated inhibition of erythropoiesis *in vitro* in a patient with type I autoimmune polyglandular syndrome and pure red blood cell aplasia. *Blood* **75,** 941.
38. Howard C.J., Sopp P., Parsons K.R. & Finch J. (1989) *In vivo* depletion of BoT4 (CD4) and of non-T4/T8 lymphocyte subsets in cattle with monoclonal antibodies. *Eur J Immunol* **19,** 757.
39. Hacker G., Kromer S., Heeg K., Ivanyi J, Wagner H. & Pfeffer K. (1992) Opportunist mycobacteria express ligands that stimulate production of human $V\gamma9$ $V\delta2$ T lymphocytes. *Infect Immun* **60,** 2753.
40. Porcelli S., Brenner M.B., Greenstein J.L., Balk S.P., Terhorst C. & Bleicher P.A. (1989) Recognition of cluster of differentiation 1 antigens by human $CD4^-$ $CD8^-$ cytolytic T lymphocytes. *Nature* **341,** 447.
41. Vidovic D., Roglic M., McKune K., Guerder S., Mackay C. & Dembic Z. (1989) Qa-1 restricted recognition of foreign antigen by a $\gamma\delta$ T-cell hybridoma. *Nature* **340,** 646.
42. Schild H., Mavaddat N., Litzenberger C. *et al.* (1994) The nature of major histocompatibility complex recognition by $\gamma\delta$ T cells. *Cell* **76,** 29.
43. O'Brien R.L., Happ M.P., Dallas A., Palmer E., Kubo R. & Born W.K. (1989) Stimulation of a major subset of lymphocytes expressing T cell receptor $\gamma\delta$ by an antigen derived from *Mycobacterium tuberculosis*. *Cell* **57,** 667.