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Cross-talk between γδ T lymphocytes and immune cells in humoral response

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SUMMARY

The role of γδ T cells in immunoregulation is largely unknown. In the current study we noted that γδ T cells play a positive role in the humoral response. These positively acting γδ T cells are required for the successful adoptive cell transfer of the humoral response, as well as for *in vitro* generation of plaque-forming cells (PFC). The presented results show that γδ T cells cause an increase in interleukin-10 (IL-10) production, which partly elucidates the mechanism of action of these cells. However, experiments with cell culture inserts strongly suggest that direct cell–cell contact between immune and γδ H-2-compatible regulatory T cells is critical to the exertion of the positive immunoregulatory function of γδ cells. The mechanism of cross-talk between these two cell populations is still not clear but we regard as most likely that the positively acting γδ T cells may interact with a complex of heat-shock protein–non-polymorphic MHC (IB) on the surface of T helper type 2 and/or B cells. This could provide, by direct cell–cell contact, the cognate recognition between γδ T-cell receptors and heat-shock protein–MHC that leads to positive internal signalling in the immune cells.

INTRODUCTION

There are two major kinds of adaptive immune responses. The first class of adaptive immune response is mediated by antigen-specific T-cell receptor (TCR) $\alpha\beta^+$ $CD4^+$ (delayed-type hypersensitivity, DTH) or $CD8^+$ (T-cell-mediated cytotoxicity) lymphocytes and is induced by intracellular pathogens. The second type of adaptive immune response is the humoral immune response mediated by antibodies produced by B lymphocytes. The main function of the humoral response is to destroy extracellular micro-organisms and prevent the spread of intracellular infection.

For complete health, every living being must be continuously protected from infection and tumours by their immune system. At the same time, there must be some mechanisms protecting organisms from development of inappropriate immune responses that are harmful to their own bodies (allergy, autoimmunity). This points to the importance of the balance of the immune response and its tight regulation by positive and negative signals.

There are many reports that humoral responses are negatively regulated by T suppressor cells[1–3] and positively regulated by T antisuppressor cells.[4] Our previous studies suggest that T $\gamma\delta^+$ lymphocytes play an important positive immunoregulatory role in the DTH response, where they are necessary for optimal adoptive transfer of DTH.[5–7] In our other studies we found that γδ T cells can down-regulate DTH in an antigen-specific manner.[8,9] These findings prompted us to determine whether T helper type 1 (Th1) cells that mediate DTH are the sole target of γδ T regulatory cells or whether Th2 and/or B cells that mediate humoral responses can also be regulated by this unique cell population.

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MATERIALS AND METHODS

Mice

Six- to seven-week-old CBA/J ($H\text{-}2^k$) and BALB/c ($H\text{-}2^d$) male mice were obtained from Jackson Laboratories, Bar Harbor, ME, or from the breeding unit of the Department of Immunology, Jagiellonian University, College of Medicine, Kraków, Poland.

Reagents

Sheep red blood cells (SRBC) were used as an antigen. These were obtained from a single animal (Colorado Serum Company, Denver, CO or from the Institute of Sera and Vaccines, Kraków, Poland). Guinea-pig serum was used as a source of complement for the development of haemolytic plaques [plaque-forming cell (PFC) assay]. Low-toxicity rabbit complement (RC) was obtained from Pel-Freez, Brown Deer, WI, and was used in experiments where lymphocytes were negatively selected by successive treatment with anti-γδ monoclonal antibody (mAb) and RC′, Protein A (Pharmacia Fine Chemicals, Piscataway, NJ) and Sepharose 4 Fast Flow

(Pharmacia LKB Biotechnology AB, Uppsala, Sweden). Anti-hamster IgG antibody-coated magnetic beads (1-μm iron magnetic particles; Advanced Magnetics, Inc., Cambridge, MA) were obtained from the manufacturer.

In some experiments, isolated cell populations were cultured in 1-μm 24-well Biocoat cell culture inserts (Collaborative Biomedical Products, Two Oak Park, Bedford, MA).

Monoclonal antibodies and cytokine standards

The following mAb were used: culture supernatants: anti-TCR γδ (hamster clone UC7-135D5) from Dr J. Bluestone, University of Chicago, IL,[10] anti-TCR γδ (hamster clone GL3) from Dr Leo Lefrancois, University of Connecticut.[11] These IgG κ antibodies, both specific for the δ-chain, belong to different subclasses of American hamster immunoglobulins, UC7 to group 3 and GL3 to group 2 (Pharmingen Research Products Catalogue); rat anti-mouse interleukin-2 (IL-2) mAb, biotinylated rat anti-mouse IL-2 and biotinylated rat anti-mouse IL-10 (Pharmingen, San Diego, CA), rat anti-mouse IL-10 (Genzyme, Cambridge, MA). In some experiments normal hamster IgG purified on a Protein A column was used as a control.

As cytokine standards recombinant mouse IL-2 (rIL-2) (Pharmingen, San Diego, CA) and rIL-10 (Genzyme, Cambridge, MA) were used. Horseradish peroxidase-conjugated streptavidin was purchased from Vector Laboratories, Burlingame, CA.

Adoptive cell transfer to antibody response

CBA/J mice were immunized by a single intravenous (i.v.) injection of 3×10^7 SRBC. Their spleens were collected 7 days later and used as a source of immune cells. Then, 7×10^7 untreated spleen cells (positive control) were transferred i.v. into naive irradiated recipients [mice received 5·85 Gy (600 rads) from an X-ray source 1 day before cell transfer]. In some experiments immune cells were treated with anti-γδ mAb alone, anti-γδ mAb + RC′ or hamster IgG + RC′ before cell transfer.

In other experiments spleen cells were separated into $\gamma\delta^+$ and $\gamma\delta^-$ cell fractions with the use of magnetic beads as described below. The resultant cell populations were transferred into naive irradiated mice. All recipient mice received also 5×10^7 SRBC on the day of cell transfer. A negative control group consisted of X-irradiated mice that received SRBC without immune cells. Seven days later, spleens were harvested and tested for a number of antibody-producing cells (PFC) in a Cunningham plaque-forming cell assay.[12]

Test for antibody production in vitro

CBA/J mice were immunized as described above and their spleens were used as a source of immune cells. Immune cells were cultured *in vitro* following the Mishell–Dutton procedure; 1×10^6 immune cells were incubated with 1×10^6 SRBC in 24-well plates for 4 days in RPMI-1640 supplemented with 10% fetal calf serum (FCS), containing sodium pyruvate, L-glutamine, solution of non-essential amino acids and 2-mercaptoethanol (2-ME). In some experimental groups immune cells were separated into $\gamma\delta^+$ and $\gamma\delta^-$ cell populations with the use of magnetic beads before culture *in vitro* with antigen. When the mixtures of $\gamma\delta^-$ and $\gamma\delta^+$ cells were cultured together, the latter formed one-tenth of the total cell population. All experimental groups consisted of three individual wells. At the end of culture, supernatants were discarded and the resultant cells were washed once and resuspended in 1 ml of RPMI-1640. Cell suspensions were then tested for number of antibody-producing cells with the use of haemolytic plaque assay.

Plaque assay

The number of antibody-forming cells was determined by the Cunningham modification of the Jerne plaque assay using guinea-pig complement.[12] The mean number of PFC was calculated from triplicate cultures or five or six mice and expressed as PFC/10^6 spleen cells or cultured spleen cells.

Magnetic bead cell separation

Immune spleen cells (5×10^8) were incubated with 50 ml of anti-γδ mAb (UC7) supernatant on ice for 40 min. Then the anti-γδ-coated cells were washed three times with phosphate-buffered saline (PBS), and resuspended in 50 ml PBS + 1% FCS, containing goat anti-hamster IgG-coated magnetic beads at five to ten beads per target cell.

Then the cell and bead mixture was incubated in a vertical 50 ml tissue culture flask on ice. After 30 min, a magnet (Advanced Magnetics, Cambridge, MA) was applied to one side of the flask. Ten minutes later, magnetic-bead-non-adherent cells were harvested, and then the magnetic-bead-adherent cells were recovered. Fluorescence-activated cell sorter (FACS) analysis of the anti-γδ immunobead-separated subpopulations has been presented elsewhere,[7] in which we reported that the percentage of γδ cells adhering to the anti-γδ beads can vary between 70 and 95% of the total adherent cells; the rest are not specifically bound. Also, the unavoidable splenic macrophage phagocytosis of some mAb-coated γδ cells that were attached to the beads probably contributed to variability in γδ T-cell recoveries.

ELISA detection of cytokines in culture supernatants

To test the influence of γδ T cells on the cytokine production in the humoral response, supernatants from Mishell–Dutton cultures were harvested after 48 hr of culture and tested by enzyme-linked immunosorbent assay (ELISA).

Quantitative ELISA of IL-2 and IL-10 employed two different mAb. Briefly, wells of 96-well microtitre plates (Corning, NY) were coated with 1 μg/ml of capture mAb in 0·1 M $NaHCO_3$ (pH 8·3) at 4°, overnight. Following blocking with 3% dry milk in PBS at 37° for 2 hr, samples and dilutions of standard recombinant mouse cytokines, were incubated on the plate overnight at 4°. Then 0·5 μg/ml of biotinylated detection mAb to tested cytokines was applied. Subsequently horseradish peroxidase-conjugated streptavidin and substrate were added (hydrogen peroxide and *o*-phenylenediamine). The enzymatic reaction was stopped 30 min later with 3 M H_2SO_4. Optical densities (OD) were determined at 492 nm.

Statistics

All the experiments were carried out two to four times and typical results are shown in the figures. Double-tailed Student's *t*-test was used to assess the significance of differences between groups, with $P < 0{\cdot}05$ taken as a minimum level of significance.

RESULTS

Treatment of immune cells with UC7, but not with another GL3 anti-γδ mAb, impedes transfer of antibody response *in vivo*

We showed previously that immune αβ contact sensitivity (CS) effector T cells depleted of regulatory 'CS-assisting' γδ T cells cannot adoptively transfer CS into naive syngeneic recipients.[5–7] In the current experiments we investigated whether γδ T cells were required for successful transfer of the humoral response.

Immune cells were treated with anti-γδ UC7 or GL3 mAb or with medium alone and were then transferred into irradiated syngeneic mice. Recipients of immune cells received, also i.v., 5×10^7 SRBC on the day of transfer. In all experiments one group of X-irradiated mice receiving SRBC alone (without cell transfer) was added to find the background antibody production which was then substracted from each experimental group. After 7 days, spleen cells from transferred mice were tested for the number of PFC as described before.

In vitro treatment of immune cells with anti-γδ UC7 mAb and subsequent transfer of cells resulted in decrease in the number of PFC from about 300 per 10^6 spleen cells to 100 per 10^6 spleen cells, while treatment with anti-γδ GL3 was ineffective. For this reason, in all further experiments UC7 mAb was used.

In another experiment immune spleen cells were treated with anti-γδ mAb, hamster IgG, or medium alone. After incubation half of the cells from each tested group were incubated with RC′ for an additional 45 min at 37°. Then all resultant cell groups were injected i.v. into irradiated recipients that were challenged with 5×10^7 SRBC. Seven days later mice were killed and their spleen cells were tested by PFC assay.

Data presented in Fig. 1 show that both treatments of immune cells with UC7 mAb alone or UC7 mAb and C′ (Groups B and C) dramatically decreased transfer of antibody response. In contrast, treatment of immune cells with control hamster IgG with or without complement did not affect the transfer of antibody response (Groups D and E).

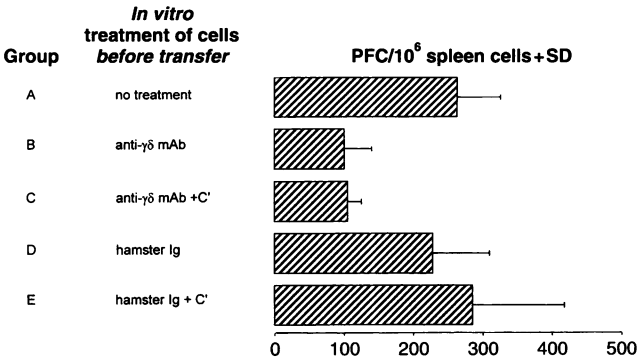

Figure 1. Treatment of immune cells with UC7 mAb impedes transfer of antibody response *in vivo*; 7×10^7 SRBC-immune spleen cells were treated with anti-γδ UC7 mAb (Group B) or hamster IgG (Group D) or medium alone (Group A) for 40 min on ice. Cells were then washed twice with PBS. After the last wash half the cells previously incubated with UC7 mAb or control IgG were incubated again with rabbit complement for 45 min at 37°. All resultant cell groups were then transferred into irradiated recipients that were challenged with 5×10^7 SRBC. In all experiments one group of mice receiving SRBC alone (without cell transfer) was added to find background antibody production which was substracted from each experimental group. Seven days after cell transfer the PFC number was tested as described in the Materials and Methods. Statistical significance: Group A versus Group B, $P < 0{\cdot}001$; Group A versus Group C, $P < 0{\cdot}001$.

Depletion of γδ$^+$ cells diminishes antibody responses *in vitro*

To confirm our findings *in vivo* (presented in Fig. 1), we tested for a role of γδ T cells in the humoral response *in vitro*. Spleen cells from SRBC-immunized mice were separated with the use of UC7 anti-γδ mAb and magnetic beads. Then the resultant cell groups were put into individual wells of a 24-well plate and cultured with SRBC for 4 days. At the end of the culture, cells were harvested and tested for antibody production by PFC assay.

Figure 2 shows that depletion of γδ$^+$ cells from immune cells significantly decreased antibody production (Group B versus Group A; positive control containing unseparated immune cells). The γδ$^+$ cells cultured with antigen did not show any production of antibody (Group C). Moreover, reconstitution of the γδ$^-$ cell fraction with γδ$^+$ cells fully restored the antibody response (Group D).

For cell co-operation, in antibody production, direct cell–cell contact and major histocompatibility complex (MHC)-compatibility (*in vitro*) are required

SRBC-immune cells were separated with magnetic beads as described before. Resultant cell populations (γδ$^+$ and γδ$^-$) were cultured separately (Groups B and F) or together (Group C). In Group E γδ$^+$ and γδ$^-$ cell populations were separated in the culture by 1-μm membranes. Therefore, under these cell-culture conditions only low molecular weight molecules (e.g. cytokines) could pass from one compartment to another and the tested cell populations could not make contact directly via their receptors (e.g. adhesion molecules, activation molecules).

To test antigen specificity of γδ T cells, γδ-depleted (γδ$^-$)

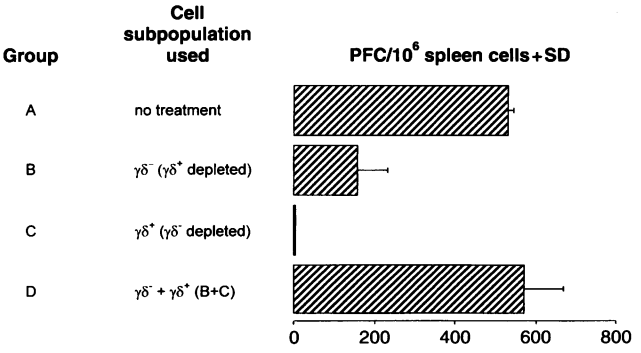

Figure 2. Depletion of γδ cells diminishes antibody responses *in vitro*. SRBC-immune spleen cells were separated into γδ$^+$ (Group C) and γδ$^-$ (Group B) cell fractions using an anti-γδ UC7 mAb and magnetic beads (for details see the Materials and Methods). Unseparated immune cells (Group A), separated (Groups B and C) and γδ$^-$ cells reconstituted with γδ$^+$ cell fraction (Group D), were then cultured with SRBC in individual wells of a 24-well plate. Four days later, cell cultures were tested in a PFC assay. Statistical significance: Group A versus Group B, $P < 001$; Group A versus Group C, $P < 0{\cdot}001$; and Group B versus Group D, $P < 0{\cdot}01$.

cells were reconstituted with γδ T cells from non-immunized MHC-compatible (CBA/J) or incompatible (BALB/c) animals (Groups D and G).

In this experiment (Fig. 3) a group of positive control (unseparated immune cells) was also included (Group A). As in previous experiments, depletion of $\gamma\delta^+$ cells from immune cells significantly decreased antibody production (Group B) in comparison to the positive control (Group A). Again, reconstitution of the $\gamma\delta^-$ cell fraction with $\gamma\delta^+$ cells almost fully restored antibody production (Group C). However, when $\gamma\delta^-$ and $\gamma\delta^+$ cells were cultured in trans-well chambers, no restoration of the antibody response was observed (Group E).

Moreover, γδ cells from MHC-compatible non-immunized mice could fully reconstitute the antibody response (Group D) while MHC-incompatible cells had no effect (Group G).

γδ T cells increase IL-10 production by immune cells in antibody response

To test an influence of γδ T cells on cytokine production in humoral response, spleen cells from SRBC-immunized mice were separated with the use of anti-γδ mAb and magnetic beads and then cultured separately or together with antigen at different cell ratios. Supernatants from Mishell–Dutton cultures were collected after 48 hr of culture and tested for IL-2 and IL-10 by ELISA.

Figure 4 shows that depletion of $\gamma\delta^+$ cells from immune cells does not influence IL-2 production in Group A versus Group B ($\gamma\delta^-$). The $\gamma\delta^+$ cell population also produced IL-2 (Group C), however, its level was much lower than the level in Groups A and B. Reconstitution of $\gamma\delta^-$ cells with $\gamma\delta^+$ cells at a ratio of 1:10 (Group D) and even at a non-physiological ratio of 1:1 (Group E) did not significantly change the production of IL-2. No influence of γδ depletion from immune cells on IL-10 production (Group D, $\gamma\delta^-$ plus $\gamma\delta^+$ cells at a ratio of 1:10, versus Group C, $\gamma\delta^-$) was observed. The $\gamma\delta^+$ cells cultured without a $\gamma\delta^-$ cell population also produced IL-10 (Group C) at levels similar to those observed in $\gamma\delta^-$ and $\gamma\delta^- + \gamma\delta^+$ cells at a ratio of 1:10. However, culture of $\gamma\delta^-$ and $\gamma\delta^+$ cells at a ratio of 1:1 (Group E) resulted in a strong increase of IL-10 production. This enhancing effect was not due to overcrowding of culture since 2×10^6 $\gamma\delta^-$ and $\gamma\delta^+$ cells used as controls produced only slightly more IL-10 as compared with 1×10^6 routinely used cells (data not shown).

DISCUSSION

There are a number of published studies dealing with the regulatory role of γδ T cells in immune responses.[2,4,13,14] Some of them clearly show a positive role of γδ T cells in cell-mediated immunity, such as an assisting role of γδ T cells in contact sensitivity.[5–7] However, studies on the positive immunoregulatory role of γδ T cells in humoral responses are rare.[4,15,16]

Our study shows, by using three different experimental approaches, that $\gamma\delta^+$ T cells play a positive role in humoral responses.

First, spleen cells from SRBC-immunized donor mice transfer a significant antibody response into X-irradiated recipients; treatment of immune cells before transfer with anti-γδ mAb + C′ (but not with control hamster IgG) results in over a 60% decrease of PFC production.

Second, depletion of $\gamma\delta^+$ cells from the immune cell population by treatment with anti-γδ mAb and anti-hamster antibody-coated magnetic beads diminishes by 70% the *in vitro* secondary immune responses by the remaining $\gamma\delta^-$ T cells. This decrease can be restored by adding back immune or non-immune MHC-compatible $\gamma\delta^+$ T cells.

Third, $\gamma\delta^-$ T cells separated from $\gamma\delta^+$ T cells in Biocoat cell culture inserts have a significantly decreased ability (by

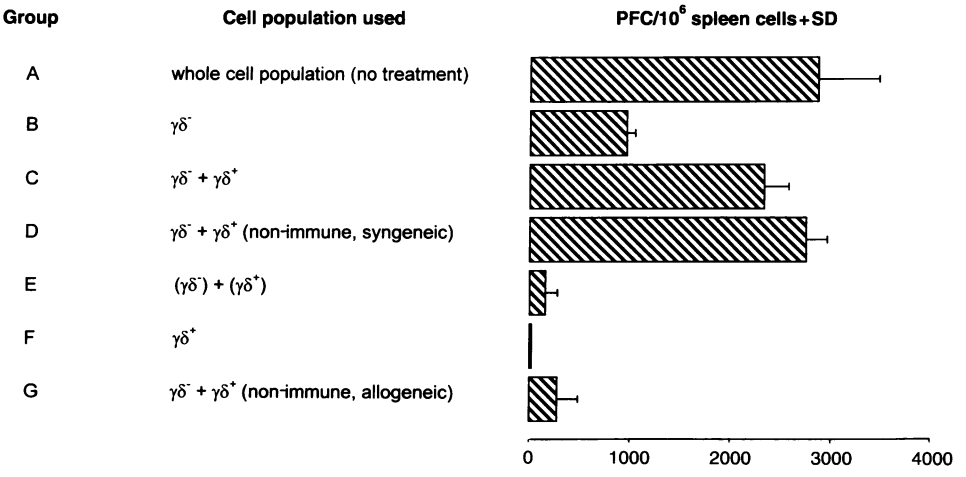

Figure 3. For full cell–cell co-operation, in antibody production direct cell–cell contact and MHC-compatibility (*in vitro*) are required. SRBC-immune cells were separated with the use of magnetic beads into $\gamma\delta^-$ (Group B) and $\gamma\delta^+$ (Group F) cell fractions. Unseparated immune cells (Group A) or $\gamma\delta^+$ and $\gamma\delta^-$ cell fractions (Groups B and F) were then cultured separately or together (Group C). In Group E $\gamma\delta^+$ and $\gamma\delta^-$ cell populations of the culture were separated in the culture by a 1-μm pore diameter membrane (trans-well chambers). To test antigen specificity γδ-depleted cells were reconstituted with MHC-compatible (CBA/J; Group D) or incompatible (BALB/c; Group G) non-immune $\gamma\delta^+$ cells. All cell groups were cultured with an antigen. Four days later all experimental groups were tested in PFC plaque assay. Statistical significance: Group A versus Group B, $P<0.02$; Group B versus Group C, $P<0.01$; Group C versus Group E, $P<0.001$; Group D versus Group G, $P<0.001$.

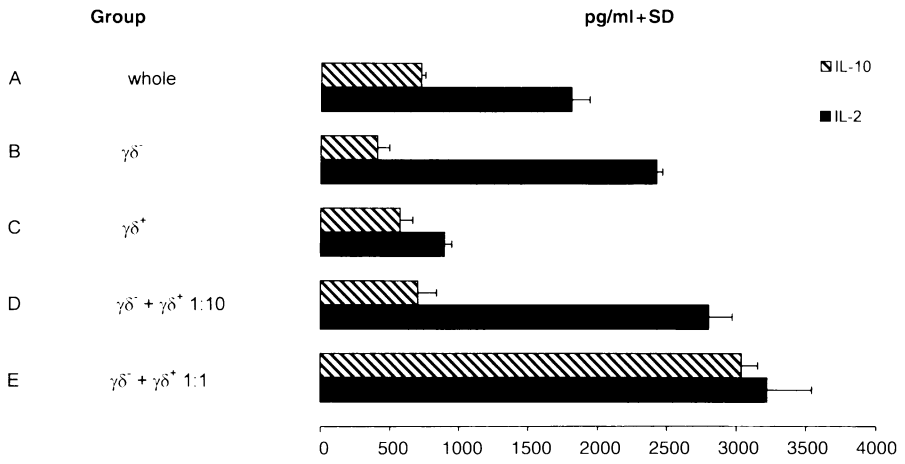

Figure 4. γδ T cells increase IL-10 production by immune cells in antibody response. SRBC-immune spleen cells were separated with the use of magnetic beads into γδ⁻ (Group B) and γδ⁺ (Group C) cell populations. Unseparated immune cells (Group A) or γδ⁺ and γδ⁻ cells (Groups B and C) were then cultured separately or together at 1:10 (Group D) or 1:1 (Group E) cell ratios. After 48 hr supernatants were collected and tested for IL-2 and IL-10 by ELISA. Statistical significance for IL-2: Group B and Group C versus Group D and E, NS. Statistical significance for IL-10: Group E versus Groups D and B, $P<0{\cdot}001$.

95%) to develop PFC as compared to these cells cultured together.

Our data strongly suggest that γδ T cells are required for successful transfer of the humoral response. A decrease of adoptive transfer in antibody response was observed when immune cells were treated before cell transfer with UC7 anti-γδ mAb (with or without complement) but not with control hamster IgG. This may suggest that cells treated with anti-γδ mAb were depleted after i.v. transfer *in vivo* by Fc receptor-bearing cells in the reticuloendothelial system of the normal recipients or by autochthonous complement. However, treatment of immune cells with another pan anti-γδ GL3 IgG mAb of different subclass had no influence on transfer of immunity. This finding is in line with our prior studies that showed the lack of biological activity of GL3 versus UC7 in *in vitro* depletion or *in vivo* stimulation of γδ CS-assisting T cells.[5,17] Other authors have also found that both these mAb differ in biological properties, such that UC7 depletes γδ T cells *in vivo*[18] while GL3 does not.[19] The significant difference in ability between the two separate anti-γδ antibodies to influence antibody response may be due to several factors, e.g. their affinity, ability to trigger complement cascade, bind to Fc receptor, or finally by the recognition of different epitopes on γδ up-regulatory cells.

Another question that we tried to answer focused on the influence of γδ T cells on cytokine synthesis during the humoral response. The presented data show that γδ T cells do not influence IL-2 production by αβ T cells *in vitro*. Similar results were found when IL-10 synthesis was tested and both γδ-depleted and γδ-reconstituted immune cells produced the same level of IL-10. However, reconstitution of γδ-depleted immune cells with γδ T cells at a non-physiological ratio of 1:1 (when γδ T cells comprise ±5% of the total cells in mouse lymphatic organs) resulted in a fourfold increase of IL-10 production.

These results, although not conclusive, may suggest that in local environments γδ⁺ T cells could contribute to enhanced IL-10 synthesis. This explanation is in line with our data, that show a significant enhancement of PFC generation by αβ⁺ T cells in the presence of γδ⁺ T lymphocytes as well as with the known immunoregulatory role of IL-10 that shifts immunity from the cellular to the humoral compartment.[20,21]

In our studies we have found that the depletion of γδ T cells from the immune cell population diminishes significantly the number of generated PFC. This decrease can be restored by adding back immune or non-immune MHC-compatible γδ⁺ T cells. Allogeneic γδ T cells were without effect. This result was in contrast to our previous studies in which we had demonstrated that in the passive transfer of contact sensitivity allogeneic γδ T cells are as good as immune or non-immune syngeneic cells in supporting the activity of αβ T effector cells.[5] The most important difference between those two experiments was in timing: the transferred CS reaction was tested within 24 hr while the Mishell Dutton culture mixture of allogeneic cells were incubated for 4 days. Therefore, we attribute, the lack of effect of γδ⁺ allogeneic cells on γδ⁻ effector cells to their functional impairment, suppression, or killing during prolonged period of incubation. To explain the mechanism of action of γδ T cells more precisely, we tested whether direct cell cell contact between immune αβ and γδ immunoregulatory cells is required. In this experiment γδ-depleted and γδ⁺ cells were separated by 1-μm Biocoat cell culture inserts which allowed for passage of soluble factors (e.g. interleukins) but did not allow direct cell cell contact. Under these conditions antibody production was reduced by 95%, which strongly suggests that γδ T cells exert their positive immunoregulatory effect on immune cells via direct cell–cell contact.

Some γδ T cells recognize peptide–MHC complexes and react to them in a conventional (αβ T-like) way yet far more preferentially recognize heat shock/stress proteins (hsp)[22,23] or non-peptide molecules (lipids, carbohydrates, phosphorylated metabolites) presented by non-polymorphic class I MHC molecules, such as TL,[24,25] Qa,[26] or CD1, or even recognize them directly.[27] The mechanisms of the cross-talk between γδ⁺ and αβ⁺ T cells are still obscure but because assisting γδ T cells are found in normal spleen, and thus before immunization, we suggested[5,7,17] that their selection is guided by

endogenous antigens, e.g. host cell surface antigens. Although the mechanisms of interactions between αβ T effector cells and γδ T regulatory lymphocytes are not clear we suppose as most probable that the positively acting γδ T cells may interact with a complex of hsp–IB on the surface of Th2 and/or B cells. This provides, by direct cell–cell contact, cognate recognition between γδ TCR and hsp–MHC that leads to positive internal signalling in the immune cells. Moreover, this may also be a signal that triggers production of IL-10.

To summarize, our experiments extend the available information on the role of $\gamma\delta^+$ T cells in immunoregulation which is still not fully understood, using *in vivo* and *in vitro* models. We also show that humoral responses are under positive control of $\gamma\delta^+$ T cells and suggest that the most probable mechanism of interaction is through recognition of hsp–IB markers on $\alpha\beta^+$ T cells by γδ T cells. Since our preliminary experiments seem to indicate that Vγ5/Vδ4 lymphocytes are responsible for this effect, our present work is aimed to elucidate these interactions more precisely.

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REFERENCES

1. McMenamin C., Pimm C., McKersey M. & Holt P.G. (1994) Regulation of IgE responses to inhaled antigen in mice by antigen-specific γδ T cells. *Science* **265,** 1869.
2. McMenamin C., McKersey M., Kuhnlein P., Hunig T. & Holt P.G. (1995) γδ T cell down-regulate primary IgE responses in rats to inhaled soluble protein antigens. *J Immunol* **154,** 4390.
3. Mengel J., Cardillo F., Aroeira L.S., Williams O., Russo M. & Vaz N.M. (1995) Anti-γδ T cell antibody blocks the induction and maintenance of oral tolerance to ovalbumin in mice. *Immunol Lett* **48,** 97.
4. Fujihashi K., Taguchi T., Aicher W.K. *et al.* (1992) Immunoregulatory functions for murine intraepithelial lymphocytes: γ/δ T cell receptor-positive (TCR^+) T cells abrogate oral tolerance, while α/β TCR^+ T cells provide B cell help. *J Exp Med* **175,** 695.
5. Ptak W. & Askenase P.W. (1992) γδ T cells assist αβ T cells in adoptive transfer of contact sensitivity. *J Immunol* **149,** 3503.
6. Askenase P.W., Szczepanik M., Ptak M., Palival V. & Ptak W. (1995) Gamma delta T cells in normal spleen assist immunized alpha beta T cells in the adoptive cell transfer of contact sensitivity. Effect of *Bordetella pertussis*, cyclophosphamide, and antibodies to determinants on suppressor cells. *J Immunol* **154,** 3644.
7. Ptak W., Szczepanik M., Ramabhadran R. & Askenase P.W. (1996) Immune or normal γδ T cells that assist αβ T cells in elicitation of contact sensitivity preferentially use Vγ5 and Vδ4 variable region gene segments. *J Immunol* **156,** 976.
8. Szczepanik M., Anderson L.R., Ushio H. *et al.* (1996) γδ T cells from tolerized αβ T cell receptor (TCR) -deficient mice inhibit contact sensitivity-effector T cells *in vivo*, and their interferon-γ production *in vivo*. *J Exp Med* **184,** 2129.
9. Szczepanik M., Anderson L.R., Ushio H. *et al.* (1997) Gamma/Delta T cells from tolerized alpha/beta-TCR-deficient mice antigen specifically inhibit contact sensitivity *in vivo* and IFN-gamma production *in vitro*. *Int Arch Allergy Immunol* **113,** 373.
10. Houlden B.A., Matis L.A., Cron R.Q. *et al.* (1989) TCR γ/δ cell recognizing a novel TL-encoded gene product. *Cold Spring Harb, Symp. Quant Biol (Immune Recognition)* **54,** 45.
11. Goodman T. & Lefrancois L. (1989) Intraepithelial lymphocytes: anatomical site, not T cell receptor from, dictates phenotype and function. *J Exp Med* **170,** 1569.
12. Cunningham A.G. & Szenberg A. (1968) Further improvements on the plaque technique for detecting single antibody forming cells. *Immunology* **14,** 599.
13. Ferric D.A., Schrenzel M.D., Mulvania T., Hsieh B., Ferlin W.G. & Lepper H. (1995) Differential production of interferon-γ and interleukin-4 in response Th1- and Th2-stimulating pathogens by γδ T cells *in vivo*. *Nature* **373,** 255.
14. Lefrancois L. & Goodman T. (1985) *In vivo* modulation of cytolitic activity and Thy-1 expression in TCR-$\gamma\delta^+$ intraepithelial lymphocytes. *Science* **243,** 1716.
15. Fujihashi K., Kiyono H., Aicher W.K. *et al.* (1989) Imunoregulatory function of $CD3^+$ $CD4^-$, and $CD8^-$ T cell. γδ T cell receptor-positive T cells from nude mice abrogate oral tolerance. *J Immunol* **143,** 3415.
16. Fujihashi K., Taguchi T., McGhee J.R. *et al.* (1990) Regulatory function for murine intraepithelial lymphocytes. Two subsets of $CD3^+$, T cell receptor-1^+ intraepithelial lymphocyte T cells abrogate oral tolerance. *J Immunol* **145,** 2010.
17. Szczepanik M., Lewis J., Geba G.P., Ptak W. & Askenase P.W. (1998) Positive regulatory γδ T cells in contact sensitivity: augmented responses by *in vivo* treatment with anti-γδ monoclonal antibody, or anti-Vγ5 or anti-Vδ4. *Immunol Invest* **27,** 1.
18. Hiromatsu K., Yoshikai Y., Matzusaki G. *et al.* (1992) A protective role of γ/δ T cells in primary infection with *Listeria monocytogenes* in mice. *J Exp Med* **175,** 49.
19. Kaufmann S.H.E., Blum C. & Yamamoto S. (1993) Cross-talk between α/β T cells and γ/δ T cells *in vivo*: activation of α/β T cells responses after γ/δ T cell modulation with the monoclonal antibody GL3. *Proc Natl Acad Sci USA* **90,** 9620.
20. Go N.F., Castle B.E., Barrett R. *et al.* (1990) Interleukin-10, a novel B-cell stimulatory factor: unresponsiveness of x-chromosome linked immunodeficiency B cell. *J Exp Med* **172,** 1625.
21. Fiorentino D.F., Bond M.W. & Mosmann T.R. (1989) Two types of mouse helper T cells. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* **170,** 2081.
22. Born W.K., O'Brien R.L. & Modlin R.L. (1991) Antigen-specificity of γδ T lymphocytes. *FASEB J* **5,** 2699.
23. Born W., Hall L., Dallas A. *et al.* (1990) Recognition of a peptide antigen by heat shock reactive γδ T lymphocytes. *Science* **249,** 67.
24. Bonneville M., Ito K., Krecko E.G. *et al.* (1989) Recognition of a self major histocompatibility complex TL region product by γδ T cell receptors. *Proc Natl Acad Sci USA* **86,** 5928.
25. Houlden B.A., Matis L.A., Cron R.Q. *et al.* (1989) A TCR γδ cell recognizing a novel TL-encoded gene product. *Cold Spring Harb, Symp. Quant Biol* **54,** 45.
26. Vidovic D., Roglic M., McKune K., Guerder S., MacKay C. & Dembic Z. (1989) Qa-1 restricted recognition of foreign antigen by a γδ T cell hybridoma. *Nature* **340,** 646.
27. Kaufmann S.H.E. (1996) γ/δ and other unconventional lymphocytes: what do they see and what do they do? *Proc Natl Acad Sci USA* **93,** 2272.