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TCRαβ+ CD8+ and TCRγδ+ Lymphocytes Inhibit the Capability of Peritoneal Macrophages to Induce Contact Hypersensitivity*

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Macrophages (Mf) play an important role in induction and regulation of the immune response. It was shown previously that subcutaneous injection of hapten conjugated macrophages (TNP-Mf) induces the contact hypersensitivity (CHS) response, whereas intravenous (i.v.) or intraperitioneal administration of TNP-Mf results in unresponsiveness as a result of induced T suppressor (Ts) cells. The aim of this study was to determine if different T cell populations influence macrophages to become inducers of immunological suppression. Our findings show that indeed i.v. injection of TNP labeled macrophages isolated from control mice into syngenic recipients induces unresponsiveness. However, i.v. administration of TNP substituted macrophages isolated from TCRα-/-, TCRδ-/- and β_2m-/- mice induces strong CHS similar to that observed after skin painting with TNP-Cl. Moreover, it was shown that TNP conjugated macrophages isolated from CD1d-/- mice were still able to promote immunosuppression when injected intravenously. This suggests that TCRαβ+ CD8+ and TCRγδ+ lymphocytes stimulate macrophages to induce immunosuppression instead of a strong CHS reaction, whereas CD1d dependent NKT cells are not involved in negative regulation of macrophage function.

Key words: Peritoneal macrophages, contact hypersensitivity response, suppression, lymphocytes.

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Macrophages play a crucial role in immunity (e.g., STOY 2001). Due to their heterogeneity, macrophages have the ability to reside in many different body tissues. These cells can be found particularly in the lymphoid organs, peritoneal cavity, lungs and in the lamina propria of the gut.

Macrophages assigned as tissue residents play a broad homeostatic role in the clearance of apoptotic and necrotic cells as well as any invading pathogens (GORDON & TAYLOR 2005). These cells are equipped to sense invading pathogens by recognition of pathogen-associated molecular patterns (PAMP). A fundamental role in pathogen recognition is played by pattern recognition receptors (PRR), e.g. Toll like receptors (TLR) broadly expressed by macrophages and other antigen presenting cells (MEDZHITOV & JANEWAY 2002). Macrophages after antigen uptake and PAMP recognition become activated and can then instruct T cells of the adaptive immune system as to the nature of the pathogen through the expression of CD80 and CD86 costimulatory molecules and by producing cytokines to direct the response (MAJEWSKA & SZCZEPANIK 2006). Therefore, macrophages play an important role both in the induction of the adaptive immune response and in the effector phase of cell mediated immunity by killing invading microbes. It is worth emphasizing that macrophages, apart from induction of immunity (SZCZEPANIK *et al.* 1993), may induce immunosuppression under some circumstances (PTAK *et al.* 1986; PTAK *et al.* 1980).

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Contact hypersensitivity (CHS) response is an example of a cell mediated immune response that can be easily induced by skin exposure to reactive haptens, e.g. picryl chloride (TNP-Cl) (CAMPOS *et al.* 2003; ITAKURA *et al.* 2005). On the other hand, CHS can also be induced after subcutaneous (s.c.) injection of hapten-labeled macrophages (SZCZEPANIK *et al.* 1993). However, intravenous (i.v.) or intraperitoneal (i.p) injection of hapten conjugated macrophages, apart from induction of T effector cells, induces T suppressor (Ts) cells as well (PTAK *et al.* 1986; PTAK *et al.* 1980). Thus, this protocol of immunization results in a status of unresponsiveness.

It should be pointed out that macrophages not only influence the behavior of T lymphocytes but also are under the influence of T cells. So, it is possible that some T cell populations might instruct macrophages to induce unresponsiveness, whereas other T lymphocytes educate macrophages to induce strong immune response, e.g. contact hypersensitivity.

The aim of this paper was to determine if different T cell populations indeed influence macrophages to become inducers of immunological unresponsiveness.

Material and Methods

Mice

Six-eight week old male CBA/J (H-2^k), B10.PL (H-2^u) and knockout mice TCRα-/-, TCRδ-/-, β_2m-/- and CD1d-/- ** on B10.PL background were used. All the mice were from the breeding unit of the Department of Human Developmental Biology, Jagiellonian University, School of Medicine. Mice were fed autoclaved food and water and kept under sterile conditions. All procedures were approved by the Ethics Committee for Animal Research in Kraków.

Reagents

Trinitrophenyl chloride (TNP-Cl) (Chemica Alta, Edmonton, Canada); paraffin oil Markol 52 (Exxon Corp., New York, NY, USA); trinitrobenzene sulphonic acid (TNBSA) (Eastman Kodak, Rochester, NY, USA).

Induction, isolation and hapten conjugation of peritoneal exudate macrophages

CBA/J, B10.PL or knockout mice (TCRα-/-, TCRδ-/-, β_2m-/- and CD1d-/- on B10.PL background) were injected i.p. with 2 ml of Markol 52 oil. Peritoneal macrophages (Mf) were harvested 4 days later and after thorough washing with 5 ml of ice cold PBS containing 5 U heparin/ml. Cells were washed, centrifuged and red blood cells were lysed with Gay's buffer and then washed with PBS. Then, peritoneal Mf were conjugated with TNP as described previously (10 mM TNBSA in PBS per $1.5x10^8$) cells (PTAK *et al.* 2000).

Induction of contact hypersensitivity

$6x10^6$ TNP conjugated macrophages (TNP-Mf) were injected i.v. into naïve recipients. When CBA/J wild type mice were used as a source of peritoneal macrophages, hapten conjugated Mf were transferred into CBA/J recipients. In experiments where Mf were isolated from knockout mice, TNP-Mf were transferred into B10.PL recipients. Seven days later, mice were challenged on both sides of the ears with 10 μl of 0.4% TNP-Cl in an olive oil-acetone mixture (1:1). The subsequent increase in ear thickness was measured 24 h later with an engineer's micrometer (Mitutoyo, Tokyo, Japan) and expressed in units of 10^{-2} mm ± SD (PTAK *et al.* 1992; PTAK *et al.* 2000). Additionally, in each experiment there was a group of litter-mate non-sensitized animals that were similarly challenged and this group served as a negative control. Each experimental and control group consisted of 5-6 mice.

TNP-Cl sensitization

Additionally, CHS was induced by skin painting with TNP-Cl. Mice were actively sensitized by topical application of 0.15 ml of 5% TNP-Cl in an acetone-ethanol mixture (1:3) to the shaved abdomen and hind feet. Control mice were shaved and painted with an acetone-ethanol mixture alone as a sham sensitization. Four days later, mice were challenged with TNP-Cl and tested as described above.

Statistics

The paired two-tailed Student's *t*-test was used with $P<0.05$ taken as the level of significance.

** TCRα-/- mice – mice that lack TCRαβ T cells, TCRδ-/- mice – mice that lack TCRγδ T cells, β_2m-/- mice – mice that lack CD8 T cells and CD1d -/- mice – mice that lack CD1-restricted NKT cells.

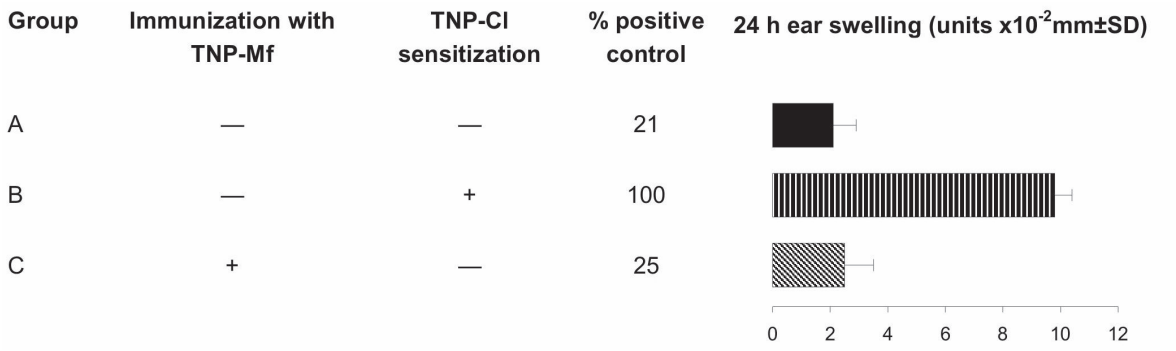

Fig. 1. Tolerance was induced by intravenous immunization with TNP conjugated macrophages. Peritioneal macrophages were isolated from naïve CBA/J mice and then conjugated with TNP. After that cells were i.v. injected into naïve CBA/J recipient mice which were challenged with TNP-Cl seven days later (Group C). TNP-Mf did not induce contact hypersensitivity. As a positive control TNP-Cl actively immunized and challenged mice were used (Group B). Mice in negative control were only challenged with TNP-Cl (Group A).
Statistical significances: Group B vs A P<0.001; Group C vs B P<0.001.

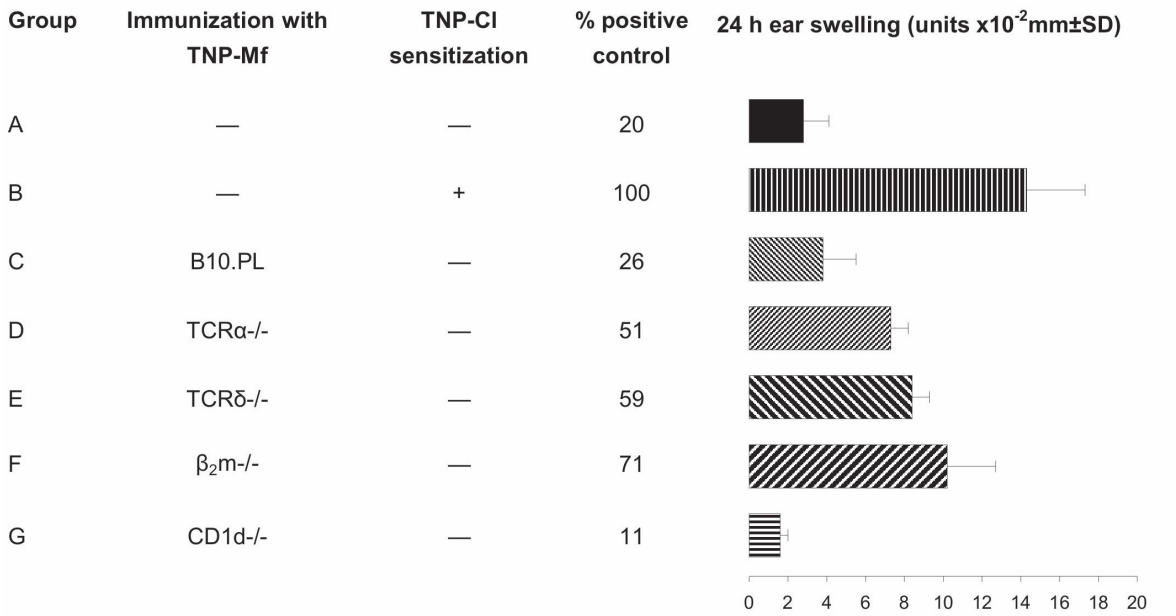

Fig. 2. Macrophage activity was negatively regulated by both TCRαβ+ CD8+ and TCRγδ+ lymphocytes. Macrophages were isolated from control B10.PL mice or following knockout mice: TCRα-/-, TCRδ-/-, β_2m-/- and CD1d-/-. Then macrophages were conjugated with TNP and i.v. injected into naïve B10.PL recipient mice. TNP-Mf from B10.PL induced tolerance instead of immunity (Group C). TNP-Mf from TCRα-/- (Group D), TCRδ-/- (Group E) and β_2m-/- (Group F) induced strong CHS reaction whereas TNP-Mf isolated from CD1d-/- mice did not induce CHS (Group G). As a positive control TNP-Cl actively immunized B10.PL mice were used (Group B). Mice in negative control were only challenged with TNP-Cl (Group A). Statistical significance: Group B vs A P<0.001; Group C vs B P<0.001; Groups D, E and F vs C P<0.01; Group G vs C p=NS.

Results

Both TCRαβ+ CD8+ and TCRγδ+ lymphocytes inhibit the capability of peritoneal macrophages to induce contact hypersensitivity. Data presented in Figure 1 show that TNP-Mf injected i.v. into naïve syngeneic CBA/J recipient mice do not induce contact hypersensitivity (Group C vs A). This result is in line with our previous experiments showing that observed unresponsiveness after i.v. injection of hapten conjugated macrophages is a result of excessively induced CD8+ T suppressor cells (PTAK *et al.* 1992; SZCZEPANIK *et al.* 1993). As a positive control TNP-Cl actively immunized mice were used (Group B).

In the next experiment we intended to test if different T cell populations are responsible for the disability of peritoneal macrophages to induce CHS. We solved this problem by employing knockout mice selectively lacking defined T cell populations. Figure 2 shows that TNP-Mf from B10.PL, similarly to hapten conjugated Mf from CBA/J mice, induce tolerance instead of immunity (Group C vs B). On the other hand, TNP-Mf from

TCRα-/- (Group D), TCRδ-/- (Group E) and β_2m-/- (Group F) induced a strong CHS reaction when i.v. injected into naïve B10.PL recipient mice. However, TNP-Mf isolated from CD1d-/- mice did not induce CHS (Group G vs A). As a positive control TNP-Cl actively immunized B10.PL mice were used (Group B).

Discussion

Contact hypersensitivity (CHS) is an example of a delayed-type hypersensitivity (DTH) induced by haptens that conjugate to self-protein Ags and peptides in the skin. CHS is mediated by local extravascular recruitment of Ag-specific circulating CS effector T cells that cause inflammatory tissue swelling, peaking 24 h after secondary skin Ag challenge. Previously we showed that apart from T effector cells, macrophages and neutrophils, other cells of the innate immunity are involved in CHS. As shown by our experiments, an early 2-h initiating response is required to locally recruit sensitized T cells. This initiating response is due to the binding of Ag to specific IgM antibodies produced rapidly after immunization by B-1 B cells (TSUJI *et al.* 2002) activated by IL-4 produced by iNKT cells (CAMPOS *et al.* 2003). As mentioned in the Introduction, CHS can be induced not only by skin painting with haptens but by s.c. injection of hapten conjugated Mf as well. On the other hand, hapten labeled macrophages injected intravenously or intraperitonealy induce immunosuppression. It is still not clear why i.v. or i.p. injection of hapten tagged Mf e.g. TNP-Mf instead of strong CHS induce unresponsiveness.

We hypothesize that different T cell populations might influence the behavior of macrophages. As a consequence of this influence, macrophages may become inducers of CHS T effector cells or T suppressor cells. To confirm our hypothesis we employed knockout mice lacking different T cell populations. Our results reveal that TNP-Mf from B10.PL control mice, similarly to TNP-Mf from CBA/J mice, induce unresponsiveness when injected i.v. into naïve syngeneic recipients. However, i.v. injection of TNP substituted Mf isolated from TCRα-/-, TCRδ-/- and β_2m-/- mice induced strong CHS similar to that observed after skin painting with TNP-Cl. These data proove that in the absence of TCRαβ+, TCRγδ+ or CD8+ lymphocytes, macrophages possess the ability to induce a strong immune response. This suggests that TCRαβ+ CD8+ and TCRγδ+ lymphocytes instruct macrophages to become inducers of unresponsiveness instead of being professional antigen presenting cells that prime T effector cells of CHS. Additionally, these data may suggest that the negative influence of T cells on macrophage function requires the parallel action of TCRαβ+ and TCRγδ+ lymphocytes, in which the action of TCRαβ+ cells is regulated by TCRγδ+ lymphocytes. This observation was made in previous work showing thatTCRαβ+ cells are indeed under the "supervision" of TCRγδ+ regulatory lymphocytes (PTAK *et al.* 1996; SZCZEPANIK *et al.* 1996, 1997; ODYNIEC *et al.* 2004). On the other hand, TNP-Mf from CD1d-/- mice after i.v. injection did not induce CHS. These data clearly show that CD1d dependant NKT cells are not involved in the negative regulation of macrophage function.

In summary, both TCRαβ+ CD8+ and TCRγδ+ lymphocytes negatively regulate the biological activity of peritoneal macrophages that instead of a strong immune response, induce a status of unresponsiveness. Further experiments are required to better understand the interplay between different T cell populations and peritoneal macrophages.

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