FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 45, No. 2, 2007
pp. 107-114

Oral β-glucan adjuvant therapy converts nonprotective Th2 response to protective Th1 cell-mediated immune response in mammary tumor-bearing mice

Jarek Baran[1,2], Daniel J. Allendorf[1], Feng Hong[1] and Gordon D. Ross[1]

[1]Tumor Immunobiology Program, James Graham Brown Cancer Center, University of Louisville, Lousville, KY, USA
[2]Department of Clinical Immunology, Polish-American Institute of Pediatrics, Jagiellonian University Medical College, Cracow, Poland

Abstract: Beta (1-3)-D-glucans were identified almost 40 years ago as biological response modifiers that stimulated tumor rejection. *In vitro* studies have shown that β-glucans bind to a lectin domain within complement receptor type 3 (CR3), or to, more recently described dectin-1 a β-glucan specific receptor, acting mainly on phagocytic cells. In this study, we assessed the intracellular cytokine profiles of peripheral blood lymphocytes from mice bearing mammary tumors receiving i.v. anti-tumor mAbs combined or not with whole glucan particle suspension given orally (WGP, 400 μg every 24 hours). The proportions of T cells producing IL-4 and IFNγ were determined by flow cytometry. The proportion of T cells producing IL-4 was significantly higher in tumor-bearing mice not receiving β-glucan-enhanced therapy. Conversely, T cells from mice undergoing β-glucan-enhanced therapy showed increased production of the Th1 cytokine IFNγ. The switch from a Th2 to a Th1 response after WGP therapy was possibly mediated by intestinal mucosal macrophages releasing IL-12.

Key words: Beta glucan - Lymphocyte subsets - Th1 and Th2 type response - Intracellular cytokines - Macrophages

Introduction

Beta (1-3)-D-glucans are major structural components of the cell wall of fungi, yeast, and certain bacteria, consisting of 1,3-linked β-D-glucopyranosyl residues with 1,6-linked β-D-glucopyranosyl side chains of varying length and distribution frequency. Beta glucans enhance the innate immune system and are pharmacologically classified as biological response modifiers [3]. In numerous animal models, β-glucans have been shown to have broad anti-infective and anti-tumor activities, with the predominant immunopharmacological effects being the activation of neutrophils, macrophages and NK cells [11,16,24,28,30,39,40]. Large molecular weight and/or particulate glucans appear to be able to activate leukocytes directly, stimulating their phagocytic, cytotoxic and anti-microbial activities, including reactive oxygen intermediates, as well as stimulating the production of proinflammatory mediators, cytokines and chemokines (IL-1, IL-6, TNFα, IFNα and IL-8) [1,10,28,41]. After exposure to soluble β-glucans, a primed state of leukocytes is generated, enhancing their response to particulate or soluble activators [40,42,45].

Analysis of the response of human and mouse leukocytes to β-glucan has shown that the M2 integrin CR3 is primarily responsible for the high affinity binding of soluble and particulate β-glucans and the cytotoxic and phagocytic responses mediated by β-glucan-stimulated macrophages, neutrophils and NK cells [28,40,46]. More recently, in addition to CR3, dectin-1 a specific β-glucan receptor has been described [4]. Dectin-1 is expressed on macrophages, monocytes, granulocytes, dendritic cells and also on B cells and a subpopulation of T cells [37,43]. On macrophages, dectin-1 is the dominant receptor mediating the phagocytosis of yeast [15]. Although macrophages and granulocytes may also use dectin-1 to capture glucan [5,37], only CR3 with bound β-1,3-glucan triggers cytotoxic degranulation in response to iC3b (inactivated complement 3b fragment)-coated tumors

Correspondence: J. Baran, Dept. Clinical Immunology, Polish-American Institute of Pediatrics, Jagiellonian University Medical College, Wielicka 265, 30-663 Kraków, Poland; e-mail: mibaran@cyf-kr.edu.pl

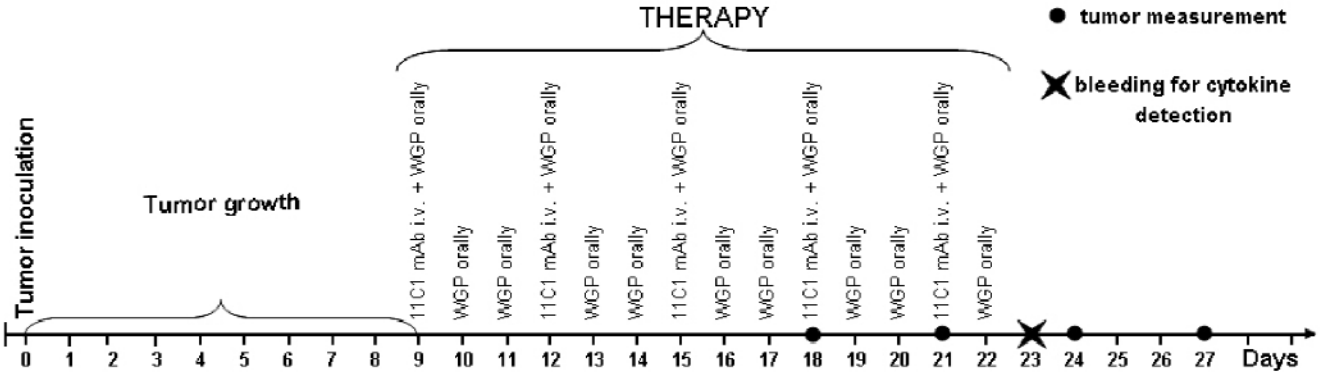

Fig. 1. Chart for WGP adjuvant immunotherapy of murine mammary tumor.

[16,40,45]. Some data also suggest that β-glucan can promote T cell-specific response [31-33,38], perhaps through triggering the secretion of proinflammatory cytokines from macrophages, neutrophils and NK cells [25,28,41]. A role for T cells in β-glucan function was also proposed because of absent tumoricidal activity in nude or T cell-depleted mice [32,33]. Previous reports had shown that there was a marked deregulation in the balance between Th1 and Th2 immune response in the course of cancer, being reported dominant Th2-type responses as a consequence of the progressive loss of Th1-type responses in tumor-bearing animals or cancer patients [22,26,34,35,48]. Because Th1 cells enhance the cytotoxic T lymphocyte (CTL) response while Th2 cells suppress it, the cytokine patterns, which reflect the Th1/Th2 balance, are crucial in mediating resistance to cancer. Cancer immunotherapy that abolishes this Th2-dominant response and promotes instead a Th1 response, would improve cancer resistance.

In our group, a tumor therapy protocol has been established in which mice first receive the anti-tumor mAbs followed by β-glucan given either intravenously or orally [16,17]. In this investigation we evaluated the intracellular cytokine profiles of peripheral blood lymphocytes from mice bearing Ptas-64 mammary tumors that were receiving i.v. mAbs and orally administrated whole glucan particles (WGP). Our results show that after treatment with WGP cytokine production by T lymphocytes was redirected from Th2 to Th1 type. This switch was mediated by intestinal mucosa macrophages which shuttled glucan particles into the lymphoid tissue and secreted IL-12 that probably stimulated naive T cells to become IFNγ+ Th1 rather than IL4+ Th2 cells.

Materials and methods

Therapeutic β-1,3-glucan. Whole glucan particles {WGP; Biothera, (formerly Biopolymer Engineering), Eagan, MN} were purified from baker's yeast through a serious of alkaline and acid extractions to yield hollow yeast cell wall ghosts composed primarily of β-1,3-1,6 glucan [18]. To remove any traces of LPS contamination, the WGP was suspended in 200 mM NaOH for 20 min at room temperature and washed several times in LPS-free water and finally in LPS-free PBS. A single-particle suspension of the material was obtained by sonication and finally, the concentration of the WGP suspension was adjusted to 4 mg/ml in PBS for storage at room temperature.

Mouse tumor model for WGP therapy. The Balb/c mammary carcinoma, known as Ptas 64 was obtained from Dr. Wei-Zen Wei (Karmonos Cancer Center and Wayne State University, Detroit, MI). This tumor line expresses a murine mammary tumor virus (MMTV) membrane antigen detectable with the 11C1 mAbs. The hybridoma producing 11C1 IgG2a anti-MMTV was generously provided by Dr. Hiroshi Fugi (Department of Molecular Immunology, Roswell Park Cancer Institute, Buffalo, NY). Four groups of five female, 6-8 week old Balb/c mice were implanted s.c. in a mammary fat pad with 0.5×10^6 Ptas-64 cells (syngeneic for Balb/c) freshly harvested from culture and suspended in 100 µl of PBS and BD Matrigel Matrix (BD Biosciences). Small, palpable tumors were allowed to form in all mice over 7-9 days before initiation of daily oral administration of WGP suspension in PBS (400 µg). Although normal Balb/c serum contains naturally occurring antibodies reactive with Ptas-64 (46), to increase iC3b deposition on the tumor cells, 200 µg of 11C1 mAbs directed against MMTV tumor antigen was given i.v. every 3 days. The therapy with 11C1 mAbs and/or WGP was carried out for a period of two weeks. Mice receiving PBS only (orally and i.v.) were used as a control group. Tumor diameter was measured every 3-rd day, and mice were sacrificed when tumor diameters exceeded 15 mm. Detailed experimental protocol is shown in Fig. 1.

Preparation of WGP-elicited peritoneal macrophages. Groups of three Balb/c or C57Bl/6 wild type mice were given i.p. a 3-ml injection of pre-warmed WGP suspension (4 mg/ml) or pre-warmed PBS (control) and peritoneal exudates rich in macrophages were harvested into PBS after 5 days. The exudates from WGP- or PBS-injected mice were pooled, washed twice and resuspended in ice-cold RPMI 1640 supplemented with 5% FBS and maintained on ice until tested.

Flow cytometry analysis of intracellular cytokines. One day after therapy was ended, groups of mice were anesthetized and exsanguinated. Blood was collected into tubes containing sodium citrate as anticoagulant. To block cytokine secretion, keeping the product within cells, peripheral blood samples were treated with 1 mg/ml of monensin (GolgiStop, BD Pharmingen). $CD4^+$ T cells were identified by cell surface staining with anti-CD3 Cy-Chrome and anti-CD4 FITC conjugated mAbs (BD Pharmingen). The red

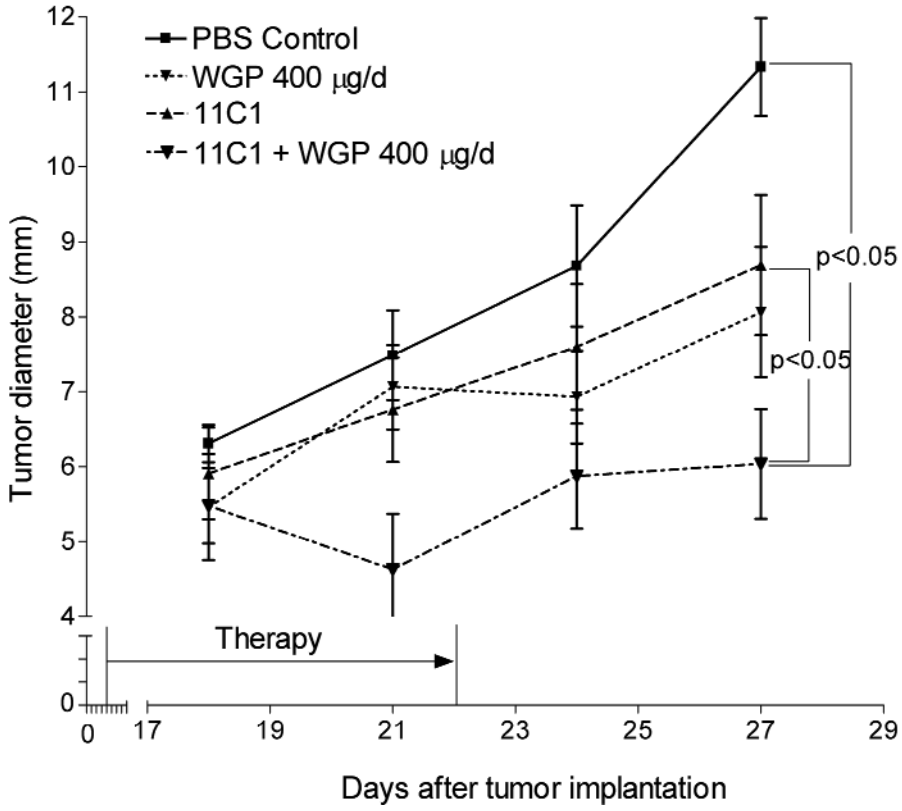

Fig. 2. Oral administration of WGP β-glucan enhances monoclonal antibody immunotherapy in mouse mammary tumor model. Four groups of 5 Balb/c mice were implanted with 0.5×10^6 Ptas64 cells subcutaneously in a mammary fat pad to form breast tumors. 7-9 days later when tumors became palpatable therapy was initiated and mice were given: I group - i.v. 11C1 mAbs, 200 μg in 100 μl PBS every third day; II group - i.v. 11C1 mAbs, 200 μg in 100 μl PBS every third day plus oral yeast glucan particles (WGP) daily for 2 weeks (400 μg/d) by intragastric injection; III group - WGP daily for 2 weeks (400 μg/d) by intragastric injection; IV group - orally and/or i.v. PBS only. Therapy was stopped on day 22 and mice were observed for tumor growth. Tumor measurements were taken over a 3-week period and mean values ±SD are presented.

blood cells were lysed, and after being washed with PBS, leukocytes were fixed and permeabilized with Cytofix/Cytoperm solution (BD Pharmingen). Then the cells were washed twice in Perm/Wash solution (BD Pharmingen) and stained for intracellular cytokines by using PE-conjugated mAbs against mouse IL-4 and IFNγ (BD Pharmingen). For tests of peritoneal macrophages, the cells from the exudates were first surface-stained with FITC-conjugated F4/80 and then fixed, permeabilized and stained for intracellular IL-12 using PE-conjugated anti-IL-12 mAbs (BD Pharmingen). To demonstrate specificity of staining, the binding of PE-conjugated anti-cytokine mAbs was blocked by preincubation the fixed/permeabilized cells with an excess of unlabeled anti-cytokine mAbs. After the cells were washed twice in Perm/Wash solution, samples were analyzed using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Palo Alto, CA) and CellQuest v3.1 software.

Statistical analysis. Average tumor size over time between groups and IL-12 production was compared and tested for significant difference by unpaired two-tailed Student's t-test. For comparison of IL-4 and IFNγ production paired two-tailed Student's t-test was applied. P value less than 0.05 was considered as statistically significant.

Results

Oral administration of WGP β-glucan enhances monoclonal antibody immunotherapy in mouse mammary tumor model

Balb/c mice were implanted s.c. in a mammary fat pad with Ptas-64 cells. After 7-9 days, when tumors became palpable, immunotherapy with 11C1 mAbs, given i.v. every three days and daily oral administration of WGP was initiated and continued for two weeks. Previous study showed that normal Balb/c serum contained naturally occurring antibodies reactive with Ptas-64 that opsonized the tumor cells growing *in vivo* with IgM, IgG, and C3 [46], and that additional i.v. injections of 11C1 mAbs produced increased surface uptake of IgG and C3 [46]. Pilot experiments demonstrated that a 200 μg i.v. dose of the 11C1 mAbs given at 3-day intervals produced a maximal coating of IgG and C3 on tumor cells (not shown). Every 3-rd day tumor diameter was measured by calipers as an average across the tumor length and width. As shown in Fig. 2 monotherapy with 11C1 mAbs or WGP elicited a considerable tumor regression (statistically not significant) but only combined therapy with both agents could arrest tumor growth. Thus oral administration of WGP β-glucan significantly enhanced immunotherapy of breast tumor in mice ($p<0.05$).

Oral WGP therapy diminishes synthesis of the Th2 cytokine - interleukin-4 (IL-4) by T cells from tumor-bearing mice

The proportion of CD3 lymphocytes producing IL-4 was detected ex vivo in peripheral blood taken from

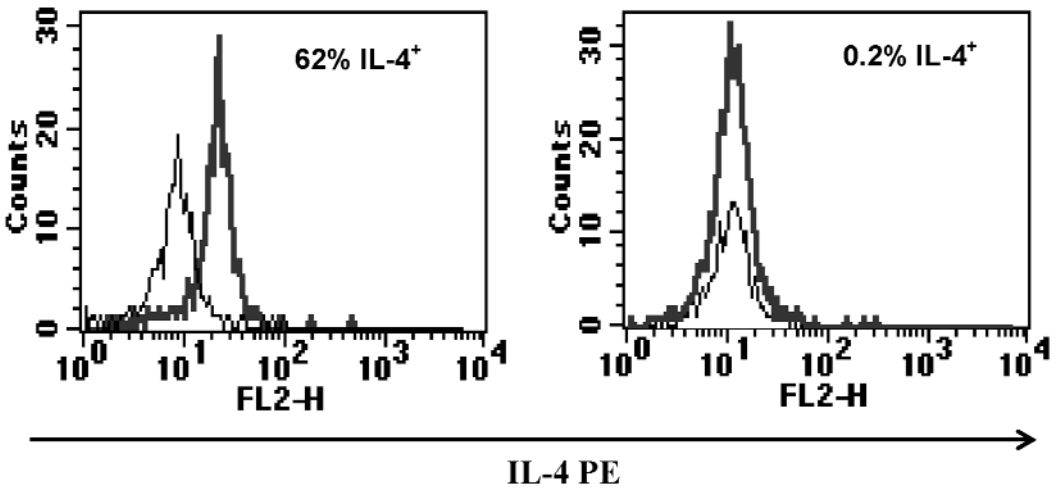

Fig. 3. Oral WGP adjuvant therapy diminishes synthesis of the Th2 cytokine - interleukin-4 (IL-4) by T cells from tumor-bearing mice. Peripheral blood leukocytes obtained from Ptas-64 tumor-bearing mice receiving 11C1 mAbs either combined (right) or not combined (left) with oral WGP adjuvant therapy were stained for the surface expression of CD3 and after being fixed and permeabilized were stained with PE-conjugated anti-IL-4 mAbs for intracellular IL-4 detection (bold line). Cells preincubated with an excess of unlabeled anti-IL-4 mAbs were used as a control (thin line). Histogram overlays show FL2 intensity corresponding to IL-4 production in the cytoplasm of T cells gated according to CD3 expression (FL3). IL-4 was detected in the cytoplasm of CD3+ T cells from tumor-bearing mice that were receiving 11C1 mAbs only (left) but no IL-4 was detected in CD3+ T cells from tumor-bearing mice receiving therapeutic mAbs in combination with WGP (right). Data obtained from 3 pooled murine bloods in each group, from one representative experiment are shown.

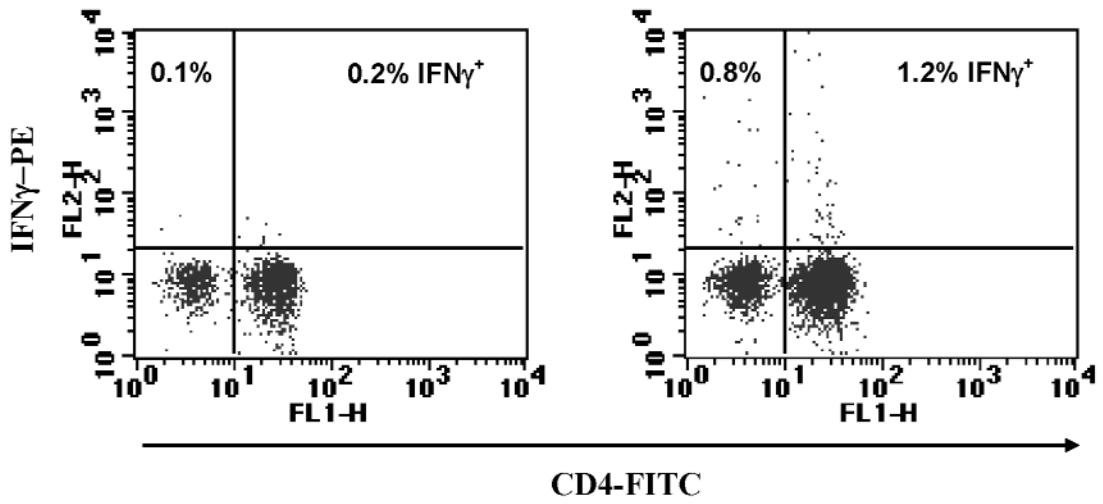

Fig. 4. T cells from tumor-bearing mice given oral WGP show increased synthesis of the Th1 cytokine - interferon gamma (IFN . Peripheral blood leukocytes obtained from Ptas-64 tumor bearing mice receiving 11C1 mAbs either combined (right) or not combined (left) with daily oral WGP treatment were stained for the surface expression of CD3 and CD4, and after being fixed and permeabilized were stained with PE-conjugated anti-IFNγ mAbs for intracellular IFNγ detection. Dot plots of CD4-FITC (FL1) vs. IFNγ PE (FL2) after gating of T cells according to CD3-CyChrome (FL3) expression are shown. Markers were set according to negative control where the binding of PE-conjugated anti-IFNγ mAbs was blocked by preincubation the fixed/permeabilized cells with an excess of unlabeled anti-IFNγ mAbs. A significant increase in the amount of T cells producing IFNγ was observed in the blood of mice receiving mAbs in combination with the oral WGP therapy. Data obtained from 3 pooled murine bloods in each group, from one representative experiment are shown. Similar pattern of staining was seen when blood from untreated vs. WGP-only treated tumor-bearing mice were analyzed (not shown).

mice bearing mammary tumors, receiving or not receiving oral WGP-enhanced immunotherapy. The proportion of IL-4 producing lymphocytes was measured by flow cytometry after cells being fixed and stained for intracellular cytokine with PE-conjugated mAbs in the presence of permeabilizing agent.

Data shown in Fig. 3 clearly demonstrate that T lymphocytes from tumor-bearing mice receiving only anti-tumor mAbs produced IL-4 (left) (mean 41.3% ± 20.5%), while additional WGP treatment almost completely reduced the amount of IL-4 positive T cells (right) (mean 0.5% ± 0.2%; $p<0.05$). Concordantly,

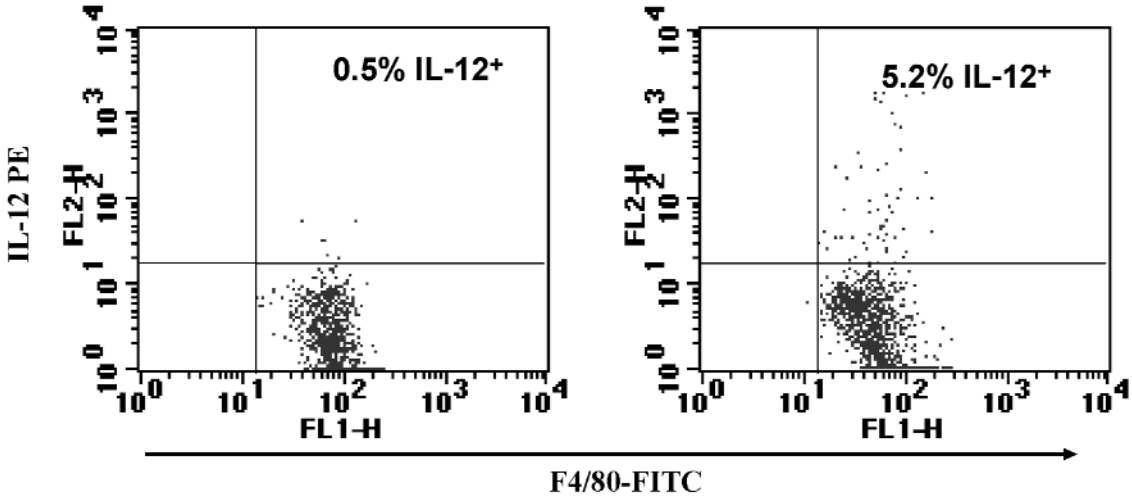

Fig. 5. Murine WGP-elicited macrophages produce IL-12 *in vivo*. Pooled peritoneal macrophages obtained from 3 i.p. PBS-injected (control) (left) or 3 i.p. WGP-injected (right) mice were stained for the surface expression of F4/80 and after being fixed and permeabilized were stained with PE-conjugated anti-IL-12 mAbs for intracellular IL-12 detection. Dot plots of F4/80 FITC (FL1) vs. IL-12 PE (FL2) after macrophage gating according to FSC/SSC are shown. Markers were set according to negative control where the binding of PE-conjugated anti-IL-12 mAbs was blocked by preincubation the fixed/permeabilized cells with an excess of unlabeled anti-IL-12 mAbs. Similar results were obtained in three independent experiments using either Balb/c or C57Bl/6 mice.

similar pattern of staining was seen when blood from untreated vs. WGP-only treated tumor-bearing mice were analyzed (not shown). There was no significant difference in the proportion of IL-4 positive T cells in mice undergoing immunotherapy alone and tumor-bearing mice not receiving any treatment (data not shown).

T cells from tumor-bearing mice given oral WGP show increased synthesis of the Th1 cytokine - interferon gamma (IFNγ)

The cytokines produced by Th1 and Th2 subpopulations have different immunological activities and the balance between the two is considered to be important to antitumor immunity. It has previously been reported that there is a marked deregulation in the balance between Th1 and Th2 in tumor-bearing animals or in cancer patients [22,26,35,48]. With this in mind, we addressed the question whether WGP treatment can convert non protective Th2 dominance to protective Th1 cellular immune response. To answer this question in the next set of experiments we investigated the proportion of T lymphocytes producing IFNγ in mice receiving immunotherapy only or immunotherapy enhanced by oral WGP treatment. To clarify which of the T cell subsets contribute to IFNγ production, in addition to anti-CD3 staining, leukocytes were stained with anty-CD4 FITC-conjugated mAbs. By this approach it was possible to show that Th1 response defined by CD4+ cells producing IFNγ was induced in mice in the course of WGP-enhanced immunotherapy (mean 0.3% ± 0.2% without vs. 1.1% ± 0.3% with WGP adjuvant immunotherapy; $p<0.05$). Moreover, IFN was also produced by CD4- T cells, presumably CD8+ cytotoxic T lymphocytes (CTL) (mean 0.1% ± 0.1% without vs. 0.6% ± 0.2% with WGP adjuvant immunotherapy; $p<0.05$) (Fig. 4). Similar amount of IFNγ-positive T cells (CD4+ and CD4-) was detected also in peripheral blood from tumor-bearing animals receiving WGP only (not shown).

Murine WGP-elicited macrophages produce IL-12 in vivo

We have shown previously that large molecular weight soluble barley and insoluble particulate (WGP) yeast β-1,3-glucans given orally, were taken up by gastrointestinal macrophages and shuttled to reticuloendothelial tissues and bone marrow, where they released small glucan fragments able to prime CR3 on the neutrophils [17]. Recently, it was also shown that orally administrated β-glucans resulted in significant increases in systemic levels of IL-6 and IL-12 [27]. The main candidates for production of these cytokines are cells of monocyte/macrophage origin. Indeed, it was reported that β-glucans activate macrophages to produce various inflammatory cytokines - IL-1α, IL-6, TNFα and IL-12 [2,12,30]. To determine whether WGP can induce IL-12 production by macrophages *in vivo*, mice were injected i.p. with WGP suspension and after 5 days WGP-elicited peritoneal macrophages were harvested and stained for intracellular production of IL-12. Membrane staining with F4/80 mAbs was performed to define macrophages in peritoneal exudates. Fig. 5

shows that peritoneal macrophages produce IL-12 after *in vivo* stimulation with WGP.

Discussion

Therapeutic efficacy of anti-tumor mAbs can be enhanced significantly by simultaneous administration of β-glucan [6,7,16,17,21,23,28,46]. Our previous studies showed that CR3+ granulocytes were the cells predominantly responsible for WGP-mediated tumoricidal activity *in vivo* [16]. In this study we demonstrate in addition, that WGP-enhanced immunotherapy of mouse mammary carcinoma converts non protective Th2 response to protective Th1 cellular immune response. This was confirmed by the findings that the proportion of CD4+ T cells producing Th1 cytokine such as IFNγ had significantly increased and that the proportion of T cells producing Th2 cytokines such as IL-4 had significantly decreased after WGP treatment.

The benefit of using β-glucans as immunotherapeutic agents for cancer treatment has been practiced in Japan since early 80's of the 20th century. Most often schizophyllan and lentinan, both β-1,3-glucans with β-1,6-branches, are used in conjunction with chemotherapy or radiotherapy. Clinical studies have demonstrated that administration of schizophyllan, an extracellular polysaccharide from *Schizophyllum commune*, along with chemotherapy, prolongs the lives of patients with lung or gastric cancers [36]. Use of lentinal, obtained from *Lentinus edodes*, an edible mushroom found in Japan, in a combined treatment of patients with advanced or recurrent gastric or colorectal cancer, has also resulted in an increased lifespan [9]. In vitro studies have revealed that these glucans activate macrophages, neutrophils and NK cells to kill sensitive tumor cells [8,19,24]. Some data also suggest that lentinan and schizophyllan can activate T cell-specific response resulting in the promotion of cytokine release [13] and potentiation of cytotoxicity mediated by CD8+ cells [38]. Indeed, neither lentinan nor schizophyllan demonstrated any anti-tumor activity *in vivo* when cyclosporine A was used as a T cell suppressor [20] or mice were T cell depleted [31]. In addition, *in vivo* lentinan application drove skewing of Th1/Th2 balance to Th1, favoring cellular immune responses in patients with digestive cancers [48]. These authors suggested, but did not provide any evidence, that lentinan-binding macrophages might produce IL-12 followed by induction of Th1 response. This hypothesis has been proven by others [25]. In this study we were able to show that murine macrophages produced IL-12 after in vivo stimulation with other β-1,3 glucan - WGP from baker's yeast. IL-12 derived from macrophages can stimulate IFNγ production from T cells, thereby favoring a Th1-pattern of response. However, the biological relevancy of this phenomenon needs to be clarified. Especially, a question needs to be answered if Th1 cytokines produced by oral WGP treatment can increase cytotoxicity of CTL *in vivo*. It is of interest as IL-2, a Th1 cytokine, represents an essential factor required for CD4+ $CD25^{hi}$ regulatory T cell (T reg) function. This subpopulation of T cells have a suppressive activity and a growing list of evidence suggest that T reg with specificity for tumor-associated antigens ameliorate immunity against a wide variety of murine and human tumors. To resolve the above issue, studies for identification and isolation of tumor-specific CTL (tetramer technology), as well as for quantitation of T reg in the blood of tumor-bearing animals are currently being performed in our group.

Despite of being crucial in mediating CTL activity, in mice Th1 cells are also involved in the differentiation of B lymphocytes and isotype switch from IgG1 to IgG2a [29]. This isotype switch is of importance, as adjuvant activity of β-glucan did not occur with a mouse IgG1 mAbs that did not activate complement but did occur with an IgM and IgG2a complement-activating mAbs [6,16]. Anti-tumor activity of β-glucan requires the tumor cells to be coated with iC3b through the action of either naturally occurring anti-tumor Abs or intravenously administered anti-tumor mAbs that activate complement. In our previous studies, tumor regression by combined mAbs and yeast β-glucan therapy was not effective in mice deficient in either C3 or CR3, highlighting the requirement for both iC3b deposited on tumor cells (by complement-activating mAbs) and its receptor CR3 on the granulocytes [16,17,46]. One can argue that Th1 cytokines, especially IL-2 (which should be increased as well by WGP), stimulate also NK cells leading to enhanced cytotoxicity without the need for antibody. A marked tumor regression in a group of mice receiving WGP only (Fig. 2) would support such scenario. However, naturally produced antibodies against MMTV (which activate complement [46]), in conjunction with WGP given orally, may be responsible for anti-tumor activity of CR3-positive cells (mainly granulocytes, but also NK cells and macrophages) and observed tumor regression. Moreover, previous data showed that beta glucan therapy of mammary tumor failed to be effective in SCID Balb/cj mice [46]. This emphasizes the crucial role for Abs in described tumor-therapy model.

Hashimoto *et al.* have suggested that large molecular weight glucans given orally are taken up by the M cells in areas of the intestine where they interact with the gut associated lymphoid tissue [14]. Our data support an active gastrointestinal (GI) uptake mechanism for glucans. Actually, we were the first to demonstrate the mechanism by which orally administered β-glucans enhance the tumoricidal activity of anti-tumor mAbs [17]. Until that, uptake and biological effects of

orally administered glucans have been highly controversial [44]. We have shown that orally administrated high molecular weight β-1,3 glucans (WGP and barley glucan) were taken up by gastrointestinal macrophages that transported them to spleen, lymph nodes and bone marrow [17]. Although we do not have any formal proof, it is tempting to speculate that in the lymphoid tissue macrophages secrete IL-12 that stimulates naive T cells to become IFNγ+ Th1 cells rather than IL4+ Th2 cells. Recently Rice *et al.* confirmed that highly purified soluble glucans interact with GI cells and enter the systemic circulation [27]. Moreover, oral administration of soluble glucans resulted in increased serum level of IL-12 in rats [27]. The route of administration of therapeutic glucans is of clinical importance as lentinan or schizophyllan, most often used in clinic, are inconvenient to administer: given i.v. daily over long periods of time, contain non-β-glucan carbohydrates and potentially allergenic proteins [6]. Free of these disadvantages is WGP. Although it has little anti-tumor activity by itself the oral administration of WGP along with antitumor mAbs given i.v. significantly enhances the efficacy of immunotherapy.

In conclusion, our data provide further evidence for the effectiveness of oral β-glucan enhanced immunotherapy of cancers. In addition to priming the CR3 of granulocytes to trigger the killing of iC3b opsonized tumor cells, β-glucan therapy can activate Th1 response favoring CTL activity. Although in many solid tumors CTL may not be able to effect tumor regression for the reason that they never actually reach tumor cells in sufficient quantities to be effective [47], but on the other hand an immunocompetent T cell component is necessary for developing anti-tumor activity [20]. Thus, targeting of tumors simultaneously with both granulocytes and CTL should result in a more effective means of eliminating tumors.

References

[1] Abel G, Czop JK. Stimulation of human monocyte beta-glucan receptors by glucan particles induces production of TNF-alpha and IL-1 beta. Int J Immunopharmacol, 1992; 14: 1363-1373

[2] Adachi Y, Okazaki M, Ohno N, Yadomae T. Enhancement of cytokine production by macrophages stimulated with (1→3)-beta-D-glucan, grifolan (GRN), isolated from Grifola frondosa. Biol Pharm Bull, 1994; 17: 1554-1560

[3] Bohn JA, BeMiller JN. (1-3)-β-D-glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohydr Polymers, 1995; 28: 3-14

[4] Brown GD, Gordon S. Immune recognition. A new receptor for beta-glucans. Nature, 2001; 413: 36-37

[5] Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S. Dectin-1 mediates the biological effects of beta-glucans. J Exp Med 197: 1119-1124

[6] Cheung NK, Modak S, Vickers A, Knuckles B Orally administered beta-glucans enhance anti-tumor effects of monoclonal antibodies. Cancer Immunol Immunother, 2003; 51: 557-564

[7] Cheung NK, Modak S. Oral (1→3),(1→4)-beta-D-glucan synergizes with antiganglioside GD2 monoclonal antibody 3F8 in the therapy of neuroblastoma. Clin Cancer Res, 2002; 8: 1217-1223

[8] Chihara G. Preclinical evaluation of lentinan in animal models. Adv Exp Med Biol, 1983; 166: 189-197

[9] Chihara G, Hamuro J, Maeda YY et al. Antitumor and metastasis-inhibitory activities of lentinan as an immunomodulator: an overview. Cancer Detect Prev, 1987; Suppl 1: 423-443

[10] Czop JK. The role of beta-glucan receptors on blood and tissue leukocytes in phagocytosis and metabolic activation. Pathol Immunopathol Res, 1986; 5: 286-296

[11] Di Luzio NR, McNamee R, Jones E, Cook JA, Hoffman EO. The employment of glucan and glucan activated macrophages in the enhancement of host resistance to malignancies in experimental animals. In: The macrophage in neoplasia. Fink MA [Ed], Academic Press, New York, 1976; pp 181-198

[12] Gantner BN, Simmons RM, Canavera SJ, Akira S, Underhill DM. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp Med, 2003; 197: 1107-1117

[13] Hamuro I, Chihara G. Lentinan, a T-cell oriented immunopotentiator. In: Immune modulation agents and their mechanisms. Fenichel RL, Chirigos MA [Eds], Marcel Dekker, New York, 1984; pp 409-436

[14] Hashimoto K, Suzuki I, Yadomae T. Oral administration of SSG, a beta-glucan obtained from Sclerotinia sclerotiorum, affects the function of Peyer's patch cells. Int J Immunopharmacol, 1991; 13: 437-442

[15] Herre J, Marshall AS, Caron E, Edwards AD, Williams DL, Schweighoffer E, Tybulewicz V, Reis e Sousa C, Gordon S, Brown GD. Dectin-1 uses novel mechanisms for yeast phagocytosis in macrophages. Blood, 2004; 104: 4038-4045

[16] Hong F, Hansen RD, Yan J, Allendorf DJ, Baran JT, Ostroff GR, Ross GD. Beta-glucan functions as an adjuvant for monoclonal antibody immunotherapy by recruiting tumoricidal granulocytes as killing cells. Cancer Res, 2003; 63: 9023-9031

[17] Hong F, Yan J, Baran JT, Allendorf DJ, Hansen RD, Ostroff GR, Cheung N-KV, Ross GD. Mechanism by which orally administered β-glucans enhance the activity of anti-tumor antibodies. J Immunol, 2004; 173: 797-806

[18] Jamas S, Rha CK, Sinskey J. Glucan compositions and process for preparation thereof. US Patent 4810646, 1989

[19] Janusz MJ, Austen KF, Czop JK. Lysosomal enzyme release from human monocytes by particulate activators is mediated by beta-glucan inhibitable receptors. J Immunol, 1987; 138: 3897-3901

[20] Kraus J, Franz G. Immunomodulating effects of polysaccharides from medicinal plants. Adv Exp Med Biol, 1992; 319: 299-308

[21] Li B, Allendorf DJ, Hansen R, Marroquin J, Ding C, Cramer DE, Yan J. Yeast beta-glucan amplifies phagocyte killing of iC3b-opsonized tumor cells via complement receptor 3-Syk-phosphatidylinositol 3-kinase pathway. J Immunol, 2006; 177: 1661-1669

[22] Maeda H, Shiraishi A. TGF-beta contributes to the shift toward Th2-type responses through direct and IL-10-mediated pathways in tumor-bearing mice. J Immunol, 1996; 156: 73-78

[23] Modak S, Koehne G, Vickers A, O'Reilly RJ, Cheung NK. Rituximab therapy of lymphoma is enhanced by orally administered (1→3),(1→4)-D-beta-glucan. Leukocyte Res, 2005; 29: 679-683

[24] Morikawa K, Takeda R, Yamazaki M, Mizuno D. Induction of tumoricidal activity of polymorphonuclear leukocytes by a linear beta-1,3-D-glucan and other immunomodulators in murine cells. Cancer Res, 1985; 45: 1496-1501

[25] Murata Y, Shimamura T, Tagami T, Takatsuki F, Hamuro J. The skewing to Th1 induced by lentinan is directed through the distinctive cytokine production by macrophages with elevated intracellular glutathione content. Int Immunopharmacol, 2002; 2: 673-689
[26] Pellegrini P, Berghella AM, Del Beato T, Cicia S, Adorno D, Casciani CU. Disregulation in TH1 and TH2 subsets of CD4+ T cells in peripheral blood of colorectal cancer patients and involvement in cancer establishment and progression. Cancer Immunol Immunother, 1996; 42: 1-8
[27] Rice PJ, Adams EL, Ozment-Skelton T, Gonzales AJ, Goldman MP, Lokhart BE, Barker LA, Breuel KF, Deponti WK, Kalbfleisch JH, Ensley HE, Brown GD, Gordon S, Williams DL. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. J Pharmacol Exp Ther, 2005; 314: 1079-1086
[28] Ross GD, Vetvicka V, Yan J, Xia Y, Vetvickova J. Therapeutic intervention with complement and beta-glucan in cancer. Immunopharmacology, 1999; 42: 61-74
[29] Schmitt E, Hoehn P, Huels C, Goedert S, Palm N, Rude E, Germann T. T helper type 1 development of naive CD4+ T cells requires the coordinate action of interleukin-12 and interferon-gamma and is inhibited by transforming growth factor-beta. Eur J Immunol, 1994; 24: 793-798
[30] Suzuki I, Tanaka H, Kinoshita A, Oikawa S, Osawa M, Yadomae T. Effect of orally administered beta-glucan on macrophage function in mice. Int J Immunopharmacol, 1990; 12: 675-684
[31] Suzuki M, Arika T, Amemiya K, Fujiwara M. Cooperative role of T lymphocytes and macrophages in anti-tumor activity of mice pretreated with schizophyllan (SPG). Jpn J Exp Med, 1982; 52: 59-65
[32] Suzuki M, Kikuchi T, Takatsuki F, Hamuro J. Curative effects of combination therapy with lentinan and interleukin-2 against established murine tumors, and the role of CD8-positive T cells. Cancer Immunol Immunother, 1994; 38: 1-8
[33] Suzuki M, Iwashiro M, Takatsuki F, Kuribayashi K, Hamuro J. Reconstitution of anti-tumor effects of lentinan in nude mice: roles of delayed-type hypersensitivity reaction triggered by CD4-positive T cell clone in the infiltration of effector cells into tumor. Jpn J Cancer Res, 1994; 85: 409-417
[34] Suzuki Y, Adachi Y, Ohno N, Yadomae T. Th1/Th2-Balancing immunomodulating activity of gel-forming (1→3)-beta-glucans from fungi. Biol Pharm Bull, 2001; 24: 811-819
[35] Tabata T, Hazama S, Yoshino S, Oka M. Th2 subset dominance among peripheral blood T lymphocytes in patients with digestive cancers. Am J Surg, 1999; 177: 203-208
[36] Taguchi T, Furue H, Kimura T, Kondo T, Hattori T, Ogawa N. Clinical efficacy of lentinan on neoplastic diseases. Adv Exp Med Biol, 1983; 166: 181-187
[37] Taylor PR, Brown GD, Reid DM, Willment JA, Martinez-Pomarez L, Gordon S, Wong SY. The beta-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. J Immunol, 2002; 169: 3876-3882
[38] Tsukada C, Yokoyama H, Miyaji C, Ishimoto Y, Kawamura H, Abo T. Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of beta-glucan. Cell Immunol, 2003; 221: 1-5
[39] Tzianabos AO. Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. Clin Microbiol Rev, 2000; 13: 523-533
[40] Vetvicka V, Thornton BP, Ross GD. Soluble beta-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. J Clin Invest, 1996; 98: 50-61
[41] Vetvicka V, Yvin JC. Effects of marine beta-1,3 glucan on immune reactions. Int Immunopharmacol, 2004; 4: 721-730
[42] Wakshull E, Brunke-Reese D, Lindermuth J, Fisette L, Nathans RS, Crowley JJ, Tufts JC, Zimmerman J, Mackin W, Adams DS. PGG-glucan, a soluble beta-(1,3)-glucan, enhances the oxidative burst response, microbicidal activity, and activates an NF-kappa B-like factor in human PMN: evidence for a glycosphingolipid beta-(1,3)-glucan receptor. Immunopharmacology, 1999; 1: 89-107
[43] Willment JA, Marshall AS, Reid DM, Williams DL, Wong SY, Gordon S, Brown GD. The human beta-glucan receptor is widely expressed and functionally equivalent to murine Dectin-1 on primary cells. Eur J Immunol, 2005; 35: 1539-1547
[44] Wu D, Han SN, Bronson RT, Smith DE, Meydani SN. Dietary supplementation with mushroom-derived protein-bound glucan does not enhance immune function in young and old mice. J Nutr, 1998; 128: 193-197
[45] Xia Y, Vetvicka V, Yan J, Hanikyrova M, Mayadas T, Ross GD. The beta-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. J Immunol, 1999; 162: 2281-2290
[46] Yan J, Vetvicka V, Xia Y, Coxon A, Carroll MC, Mayadas TN, Ross GD. Beta-glucan, a "specific" biologic response modifier that uses antibodies to target tumors for cytotoxic recognition by leukocyte complement receptor type 3 (CD11b/CD18). J Immunol, 1999; 163: 3045-3052
[47] Yeo TK, Brown L, Dvorak HF. Alterations in proteoglycan synthesis common to healing wounds and tumors. Am J Pathol, 1991; 138: 1437-1450
[48] Yoshino S, Tabata T, Hazama S, Iizuka N, Yamamoto K, Hirayama M, Tangoku A, Oka M. Immunoregulatory effects of the antitumor polysaccharide lentinan on Th1/Th2 balance in patients with digestive cancers. Anticancer Res, 2000; 20: 4707-4711

Received: September 27, 2006
Accepted after revision: December 1, 2006