Archivum Immunologiae et Therapiae Experimentalis, 2001, **49**, Suppl. 1, S13–S21
PL ISSN 0004-069X

Review

Two Receptor Theory in Innate Immune Activation: Studies on the Receptors for Bacillus Culmet Guillen-Cell Wall Skeleton

TSUKASA SEYA[1, 3]*, MISAKO MATSUMOTO[1, 3], SHOUTARO TSUJI[1, 3], NASIM A. BEGUM[1], MIDORI NOMURA[1, 3], ICHIRO AZUMA[2], AKIRA HAYASHI[1] and KUMAO TOYOSHIMA[1]

[1] Department of Immunology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Higashinari-ku, Osaka 537, Japan, [2] Hakodate National College of Technology, Tokura 14-1, Hakodate 042-8501, Japan, [3] The Organization for Pharmaceutical Safety and Research (OPSR), Tokyo 113, Japan

Abstract. Activation of the innate immune system is a prerequisite for the maturation of dendritic cells (DC) and macrophages (Mϕ) followed by clonal expansion of the lymphocytes, targeting cells expressing "non-self" antigens. Microbes usually have a component competent to activate DC/Mϕ for antigen presentation. This component has been called adjuvant, but recently renamed "pathogen-associated molecular pattern" (PAMP) or modulin based on its molecular identification. Here, we propose the hypothesis that DC/Mϕ express two sorts of receptors for PAMP, whose signaling pathways lead to a sufficient antigen (Ag)-presenting state. In bacterial infection, a Toll-like receptor (TLR) and an uptake receptor participate in DC maturation and Mϕ activation. Likewise, with a number of viruses, two of the receptors, with short consensus repeats (SCR), immunoglobulin-like domains or chemokine receptor-like motifs etc. induce functional modulation of DC/Mϕ. In immune therapy for cancer, primary activation of the innate system would be essential for tumor Ag-specific T cell augmentation. Cancer cells express tumor-associated Ag but barely co-express PAMP, which situation does not allow for the activation of innate immune responses. Supplementing tumor-associated Ag with PAMP may be an effective therapy for patients with cancer. Here, we discuss the possibility of an innate immune therapy for cancer with reference to bacillus Culmet Guillen cell-wall skeleton (BCG-CWS).

Key words: modulin; pattern-recognition receptors; leucine-rich repeat (LRR); short consensus repeat (SCR); antigen-presenting cells (APC).

Introduction

In the human immune system, lymphocytes are major effectors involved in the destruction of foreign material. Each clone of a lymphocyte expresses a specific antigen receptor which is generated by somatic gene rearrangement and is clonally distributed on T and B cells. A tremendous number of clones, each expressing unique antigen receptors can cover a huge variety of non-self antigens. In order to eliminate foreign cells, $CD8^+$ T lymphocytes mature into cytotoxic T lymphocytes (CTL) equipped with perforin, granzymes and

* Correspondence to: Tsukasa Seya, M.D. Ph.D., Department of Immunology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Higashinari-ku, Osaka 537-8511 Japan, fax/tel.: +81 6 6973 1209, e-mail: tseya@mail.mc.pref.osaka.jp

Fas ligand, while B lymphocytes mature into plasma cells to produce antibodies (Abs). Antigen (Ag) is absolutely required to trigger activation of the lymphocyte immune system, a system designated as acquired immunity[1].

Minimal as well as ineffective Ab production and CTL induction *in vitro* have resulted from stimulation with pure Ag in the absence of adjuvant[19]. Animals effectively produce Ab against foreign material, however, when they are co-sensitized with adjuvant plus purified Ag. Adjuvants, consisting of mineral oil and bacterial constituents, do not directly activate lymphocytes, but can potentially induce activation of innate immune responses, which can then lead to total immune activation involving lymphocytes. Recent findings have, in fact, shown that the target of adjuvant is indeed the innate immune system, including antigen-presenting cells (APC), leading to the hypothesis that the innate immune system must be activated prior to the priming of the lymphocyte system[11, 12]. If this is the case, lymphocyte activation is strictly controlled by the more basic innate system, which is regulated by adjuvants. However, the role of adjuvant in the innate system has not been clarified, since the innate system *per se* has not been well characterized compared with the acquired immune system[12].

Most of the known microbial products that have immunostimulatory activity represent invariant structures shared by large groups of microorganisms[27, 28] which are absolutely essential for the microbe's survival. For instance, lipoteichoic acids (LTA) and lipopolysaccharide (LPS) are common components of Gram-positive and Gram-negative bacteria, respectively. Double-stranded RNA is a structural signature of several groups of RNA viruses. Specific CpG repeats without methylation are present in bacterial DNA. Mannans and galactans are conserved as characteristic components of yeasts and bacteria. In addition, bacteria cannot survive without certain peptidoglycans, which are not shared with mammals. None of the above-mentioned molecules are found in host organisms, and all are essential for the physiology and survival of the respective microbes. Notably, these molecules exhibit potent immunomodulatory activity in the host immune system, similar to that of adjuvants, and are designated after molecular identification as pathogen-associated molecular patterns (PAMPs)[27] or modulins[17]. Based on these findings, we suggest the necessity of immune stimulation with both adjuvants (or PAMP) and Ag for the induction of strong host immune responses.

Tumor cells share the same major histocompatibility complex (MHC) as host cells and barely express PAMPs. Tumor-associated Ags, however, have been identified particularly in melanomas[34]. Thus, we hypothesized that the inability of tumors to provoke host immune responses is due to the lack of PAMPs in patients with cancer. Since PAMP-mediated immune activation proceeds presumably in a non-specific manner with inherited primary responses, we proposed that administering supplements containing PAMPs as adjuvants to patients could result in an increased efficiency of immune responses against tumor Ags. This was supported by the observation that severe infection sometimes improves the prognosis of patients with potentially lethal cancer. We have challenged cancer patients with bacillus Culmet Guillen-cell wall skeleton (BCG-CWS) for this purpose and have obtained good results[15, 16, 39]. We have designated this PAMP-supplementation therapy as "innate immune therapy for cancer".

BCG-CWS Revisited

BCG or mycobacterium infection induces potent immune responses (see below) in the host immune system. Live BCG therapy was commenced 20 years ago for treatment of bladder cancer, and has resulted in good prognosis[6, 21]. Live BCG therapy has since been accepted as an effective treatment for a variety of other cancers as well. BCG-CWS is a highly purified factor of the cell-wall skeleton of BCG. It consists of only mycolic acid, arabinogalactan and peptidoglycan, and lacks possible contaminants such as treharose dimycolate (TDM), lipoarabinomannan or phospholipids[3]. Its anti-tumor activity was first described by YAMAMURA et al.[39] in the 1960's, ten years before live BCG anti-tumor immunotherapy was initiated (Fig. 1a). The most prominent difference between live BCG and BCG-CWS treatment in tumor immune therapy indicates that the non-infectious components of BCG are sufficient to elicit anti-tumor host responses. In other words, live BCG-mediated cellular responses and latent infection are not essential to potentiate host immunity for regression of cancer in patients. This point was supported by many animal experiments using transplanted tumors in guinea pigs and mice[2, 41, 42]. Unexpectedly, however, it was suddenly designated an "ineffective" drug in the 1970's.

Revival of cancer immune therapy involving BCG-CWS has come in Japan with the clinical study by HAYASHI et al.[16] who reported an excellent 5-year sur-

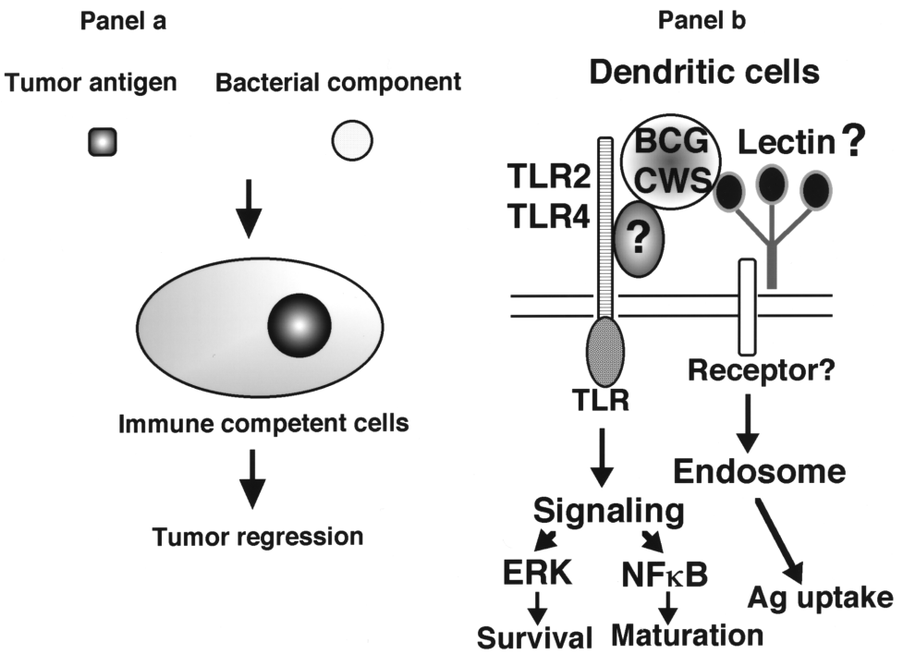


Fig. 1. Panel a – principle of innate immune therapy for cancer. Micro-organisms are often found to activate immune competent cells, which non-specifically potentiate tumor immunity if tumor Ag is provided. The exact mechanism for eliciting tumor immunity remains unknown. The non-infectious reagent, PAMP or modulin, possesses tumor-regressive activity and has been administered as an adjuvant in experimental anti-tumor therapy. Panel b – receptors for BCG-CWS. BCG-CWS is a PAMP/modulin that binds to innate immune competent cells, Mϕ/DC. Two sorts of receptors were identified on Mϕ/DC. One is Toll-like receptors (TLR), TLR2 and 4, which transduce signals through ERK and NFκB, resulting in Mϕ/DC survival and maturation. The other is a putative lectin receptor which may facilitate endosomal uptake of BCG particles

vival rate of the BCG-CWS therapy in stage III lung cancer patients. These results are intriguing, given that no other chemotherapeutic agents were used in conjunction with BCG-CWS in patients with post-surgical tumor resection[15, 16]. In contrast, patients with distant metastasis are out of the target of this therapy[16]. Surgical resection of the tumor is an essential prerequisite for this immune therapy, since the host immune system is often modulated by factors produced in tumors. In fact, serum interferon γ (IFN-γ) levels are increased by treatment with BCG-CWS in patients only after tumor elimination. In cancer patients, induction of IFN-γ may be a good marker for host immune activation[15, 16].

The results of this study are summarized in the ref.[9]. In stage III lung cancer patients, prediction of 5-year survival is 51% (Caplan-Mayer method), suggestive of BCG-CWS therapy being significantly effective compared with the conventional therapy used in our cancer institute. Based on these data, we are attempting to re-introduce BCG-CWS cancer therapy and, finally, to establish a "general" principle of tumor immune therapy.

Two Receptor Theory for PAMP: in the Case of BCG-CWS

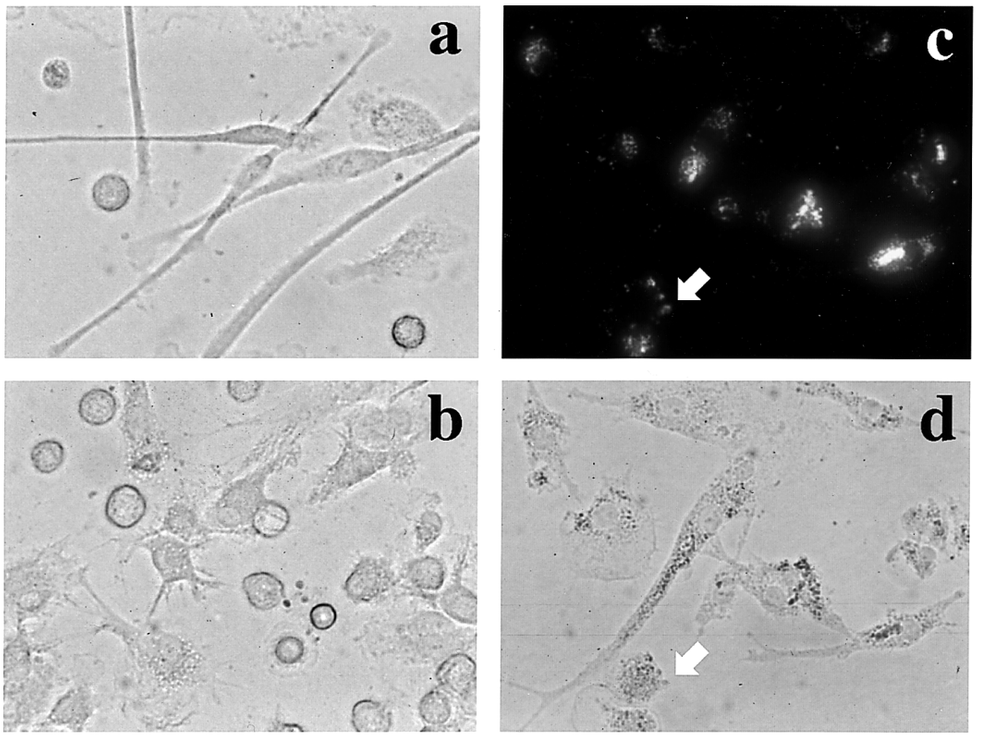
By flow cytometric analysis, FITC-labeled BCG-CWS binds monocytes, neutrophils, dendritic cells and activated macrophages but not lymphocytes[37]. Although elevation of IFN-γ is a final outcome of *in vivo* BCG-CWS treatment in cancer patients, brefeldin A-treated lymphocytes still do not induce IFN-γ[25]. Hence, the targets for BCG-CWS are not lymphocytes, including T and B cells, but rather cells of myeloid lineages. In other words, BCG-CWS serves as a modulator of the innate immune system. The target cells appear to express two sorts of receptors for BCG-CWS. One is a Toll-like receptor (TLR), which is a family of proteins with an ectodomain of leucine-rich repeats (LRR) and an intracellular IL-1R-like domain[26]. In human cells, TLR2 and TLR4 are receptors for BCG-CWS[26]. The other is a putative lectin receptor which is being identified in our laboratory (TSUJI et al., to be published elsewhere). BCG-CWS is phagocytozed through the lectin by endosomal uptake and transmits

signals through TLR2/4 (Fig. 1b). TLR are reported to induce two types of responses in dendritic cells (DC), resistance to apoptosis and maturation[32]. DC maturation is a result of nuclear factor κB(NFκB) activation, while the prolonged survival of DC is sustained by an ERK--related pathway[32]. As shown by UEHORI and TSUJI (submitted for publication), BCG-CWS consists of combinations of mycolic acid, arabinogalactan and peptidoglycan, and each component may play a respective role in the binding/activation of the two receptors on DC.

In the mouse model, BCG-CWS does not induce delayed-type hypersensitivity (DTH), unlike in humans or guinea pigs. BCG-CWS treatment caused regression of transplanted tumors in mice with much less efficiency than that seen in humans. The mouse innate immune system may differ in some respects from those of humans and guinea pigs. For instance, recent reports suggested that there are 8 CD1b and c haplotypes in guinea pigs[10] and 4 haplotypes CD1a, b, c and d in humans[7, 23], whereas mice have only one CD1 isoform, known as CD1d[20]. It has been accepted that CD1d presents αGal. Cer[20], while CD1b presents mycolic acids[5, 29]. Thus, mice lack the presenting molecule for mycolic acids. The species differences with respect to transplanted tumors and DTH induction may reflect essential differences in the innate immune systems of various species. Another possibility is that certain properties of tumor lines are an important factor for raising immune activation. The MHC level, particularly HLA-E and class I, on targets is a critical factor for discriminating between CTL-mediated killing or NK-mediated killing in the host environment[13]. BCG-CWS would be effective for tumors with high class I expression levels, whereas NK targets tumors with low MHC levels[13].

Mechanism whereby BCG-CWS Activates the Human Immune System

BCG-CWS has been identified as an adjuvant[3]. Given that adjuvant usually potentiates the innate immune responses of APC but not lymphocytes, we added BCG-CWS to each maturation step from monocytes to mature DC in an *in vitro* assay. It selectively mediated



a: BCG-CWS + GM-CSF
b: EB + GM-CSF
c: FITC-BCG-CWS (7 h)
d: FITC-BCG-CWS (7 h)

Fig. 2. Morphology of DC and phagocytosis of FITC-BCG-CWS. Immature DC differentiated with GM-CSF and IL-4 for 6 days were incubated for additional 2 days (a, b) or 7 h (c, d) in a medium containing GM-CSF (500 U/ml) and one of the following materials: a – BCG-CWS (15 μg/ml), b – emulsion buffer (E_B; 15 μl/ml), c – FITC-BCG-CWS (15 μg/ml). Magnification: x400. Cells were observed under a microscope (a, b), a light fluoresence microscope (c), or a phase-shift microscope (d) [the same field as (c)]. White arrows show FITC-BCG-CWS binding to the surface of a floating cell

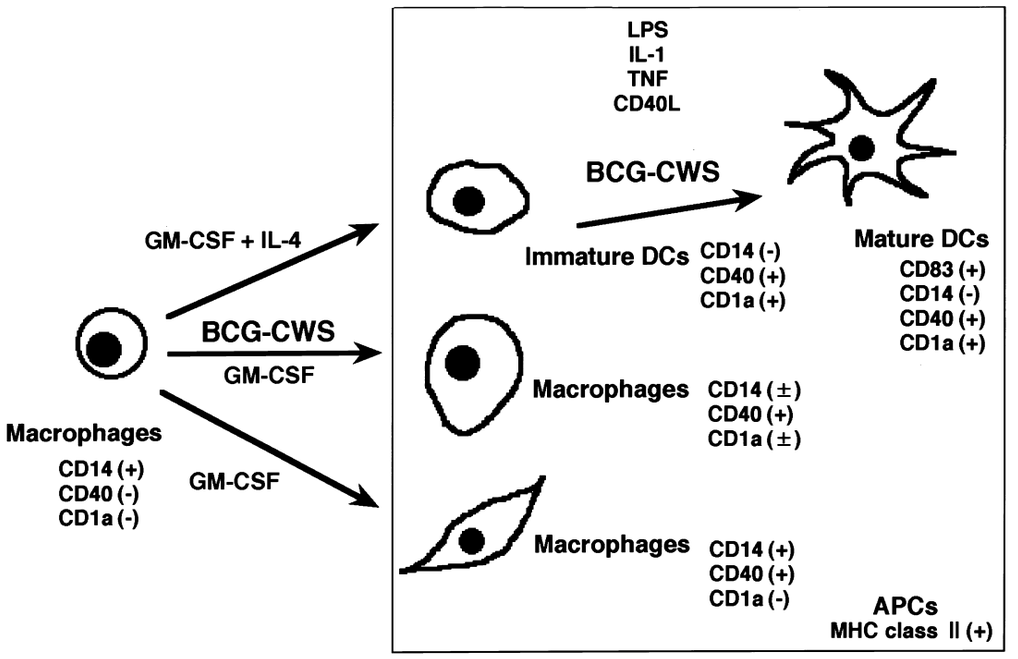


Fig. 3. Maturation of myeloid cell lineage in humans. Hierarchy of maturation of myeloid lineages. The maturation steps mediated by BCG-CWS are indicated. BCG-CWS has the ability to mature DC to an Ag-presenting form and to differentiate monocytes to activated Mϕ

the step involving the immature-to-mature conversion of DC (Fig. 2 and 3). BCG-CWS up-regulated the costimulators CD83 and CD86 as well as MHC levels and secretion of IL-12 and tumor necrosis factor α (TNF-α) by DC[37]. In addition, BCG-CWS had an ability to convert monocytes to active macrophages (Mϕ) (Fig. 3). These functional features support the hypothesis that BCG-CWS is a PAMP contributing to the final maturation of myeloid cells whose functional profile is representative of adjuvant treatment. There have been no data, however, indicating that the presentation of tumor antigen is elevated on Mϕ/DC secondary to BCG-CWS stimulation. This point needs to be clarified in APC harvested from BCG-CWS-treated patients with cancer and from BCG-CWS-treated guinea pigs inoculated with tumor cells. Mice, however, do not appear to be an appropriate animal model for testing innate immunity. Induction of tumor-specific CTL could be tested *in vitro* using tumor cells and lymphocytes from guinea pigs with syngeneic tumors.

Principle of Innate Immune Therapy for Cancer

Although the final immune responses, including CTL induction, remain to be identified, we found that, in animal systems, administration of tumor cells with microbial products resulted in significant regression of some kinds of syngeneic tumors. The microbial pro-

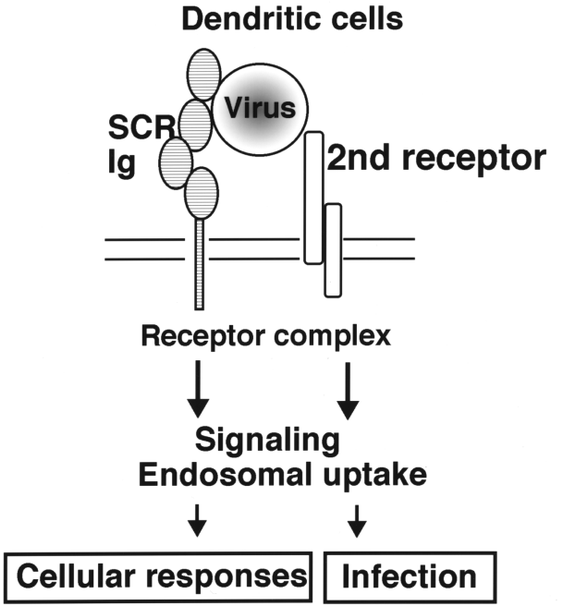


Fig. 4. Two receptors in virus infection. Two receptors on human cells are often associated with virus infection. Examples of measles virus and its receptors are shown in this figure

Table 1. Current information about human Toll-like receptors (TLR)

TLR family	Protein (LRR+TIR)	Expression in tissues	Function
TLR1	786aa	spleen, ovary, small intest, thymus, PBL, T&B cells, macrophage, DC	lipoprotein recognition??
TLR2	784aa	PBL, spleen, bone marrow, macrophage, DC IEC	PGN, lipoprotein recognition
TLR3	904aa	placenta, pancreas IEC, DC	??
TLR4	799aa	spleen, PBL, IEC, monocyte, macrophage, DC	LPS, LTA recognition
TLR5	837aa	ovary, PBL, prostate, testis	??
TLR6	796aa	thymus, ovary, lung, spleen	lipoprotein recognition??
TLR7	1049aa	lung, placenta, PBL spleen, macrophage, DC	??
TLR8	1041aa	lung, placenta, PBL spleen, macrophage, DC	??
TLR9	1032aa	spleen, PBL macrophage, DC	non-methylated CpG recognition

IEC – interstitial epithelial cells, PBL – peripheral blood lymphocytes, PGN – peptidoglycan, LTA – lipoteichoic acid

Table 2. Human membrane-associated SCR proteins

SCR protein	Mr (kDa)	Structure	Ligands	Destribution	Function
CR1 (CD35)	A: 190 B: 220 C: 160 D: 250	30 SCRs 37 SCRs 23 SCRs 44 SCRs	C3b, C4b C3bi	phagocytes podocytes (kidney) dendritic cells oocytes	immuneclearnce self-protection Ab production?
CR2 (CD21)	140	15/16 SCRs	C3dg, C3bi EBV	B lymphocytes thymocytes	Ab production EBV receptor
DAF (CD55)	70	4 SCRs	C3b, C4b some echo V adhesin *E. coli*	all cells to be protected from C	self-protection decay of convertases echo V receptor
MCP (CD46)	45~70	4 SCRs	C3b, C4b measles V streptcocci	all nucleated cells	self protection factor I-cofactor measles V receptor

ducts of bacteria, fungi and viruses predominantly bind Mϕ/DC to activate the innate immune system. Again, lymphocytes are not a target for this immune therapy. In most cases, two receptors are thought to be involved in the binding of these products and the activation of APC. For BCG-CWS, one receptor is TLR and the other is a putative lectin receptor not yet identified (Fig. 1b). With other bacterial products, such as M161Ag/MALP2[24, 36] of mycoplasma and its homologs of borrelia[18] and treponema[22], the usage of these two kinds of receptors seems to be conserved. Furthermore, it has become clear that some viruses also modulate the function of DC/Mϕ by binding two different kinds of receptors on these cells (Fig. 4). Thus, the most likely hypothesis is that the stimulation of DC by microbes participates in the initial modulation of antigen presentation of the DC followed by the expansion of targeting lymphocyte clones. The threshold level of Ag presentation by APC would be lowered in the response of APC to foreign materials, and tumor Ags could be sufficiently

presented by APC if the APC had been prestimulated with the foreign material. The signals transduced by the two receptors remain largely unknown and may play an important role in the effective presentation of tumor Ags.

Perspectives

The concept that bacterial substances ameliorate tumor regression is not new. This phenomenon was first described more than 100 years ago[9]. The foreign materials were called "adjuvant" and their potential immune activation properties have been investigated extensively[40]. However, the molecular mechanism whereby the foreign material induces tumor regression has not been elucidated. A more thorough understanding of this mechanism is now possible, however, with the identification of the TLR (Table 1) and other bacterial receptors, including lectins[14]. A variety of combinations of these receptor, families may recognize a huge number of micro-organisms. To date, limited information is available about these receptors, but they may participate in the first non-self recognition in the immune system (Table 1). We favor the interpretation that these receptors synergistically induce common cell-activation responses supported by similar signaling events. It is becoming clear that a common signal transduction pathway may be evolutionally conserved across species[31, 33] and the resulting cellular responses are aimed at the activation of lymphocytes in vertebrates. Furthermore, DC functions and maturation are found to be modulated by viruses. Viruses usually choose short consensus repeat (SCR), chemokine and Ig superfamily receptors (Table 2), e.g., HIV binds CD4 (Ig) and a chemokine receptor on Mϕ[8, 38]. Measles virus appears to use two receptors CD46 (SCR)[30] and an alternative receptor (CDw150, SLAM) on DC/Mϕ; HHV6 also use CD46 and one of the chemokine receptors[35].

Although an additional event may occur in DC which induces DC-specific responses, the functions of microbial receptors are essentially conserved across species in myeloid lineages. The anti-tumor effect of these receptors may also be conserved over species. We suggest that an initial activation of microbial receptors on APC is a prerequisite for the induction of effective tumor immunity. This hypothesis will be tested with non-mammals, since Toll SCR, lectin and Ig receptors are widely conserved from the fly to humans[28].

Dendritic cell therapy is a potentially effective treatment for cancer prognosis. Patients' monocytes are differentiated to DC with GM-CSF plus IL-4, and these DC are coated with tumor Ag which can be prepared from a resected tumor. These Ag-stimulated DC can be further matured for effective Ag-presentation with an innate immune activator, such as BCG-CWS, and these mature CD injected back into the patients. If microbial products play an important role in effective Ag presentation involving tumor-associated Ag, this therapy may result in the elimination of scattered micro metastatic tumor cells in patients. Such a study is currently under way in our laboratory.

Acknowledgment. We are grateful to Dr. H. Koyama and M. Tatsuta for the support of this work. This work was supported in part by the Organization for Pharmaceutical Safety and Research (OPSR).

References

1. ALT F. and ALLEN P. M. (1997): The recognition of antigen. In JANEWAY C. A. JR. and TRAVERS P.: Immunology. 3rd ed. Current Biology Ltd., UK, and Garland Publishing Inc., New York, USA.
2. AZUMA I., KANETSUNA F., TANIYAMA T., YAMAMURA Y. and HORI M. (1975): Adjuvant activity of mycobacterial fractions. I. Purification and *in vivo* adjuvant activity of cell wall skeletons of *Mycobacterium bovis* BCG, *Nocardia asteroides* 131 and *Corynebacterium diphtheriae* PW8. Biken J., **18**, 1–13.
3. AZUMA I., KISHIMOTO S., YAMAMURA Y. and PETIT J. F. (1971): Adjuvanticity of mycobacterial cell walls. Jpn. J. Microbiol., **15**, 193–197.
4. AZUMA I., RIBI E. E., MEYER T. J. and ZBAR B. (1974): Biologically active components from mycobacterial cell walls. I. Isolation and composition of cell wall sekleton and component P3. J. Natl. Cancer Inst., **52**, 95–101.
5. BECKMAN E. M., PORCELLI S. A., MORITA C. T., BEHAR S. M., FURLONG S. T. and BRENNER M. B. (1994): Recognition of a lipid antigen by CD1-restricted $\alpha\beta^+$ T cells. Nature, **372**, 691–694.
6. BROSMAN S. A. (1982): Experience with bacillus Calmette Guerin in patients with superficial bladder carcinoma. J. Urol., **128**, 27–30.
7. CALABI F. and MILSTEIN C. (1986): A novel family of human major histocompatibility complex-related genes not mapping to chromosome 6. Nature, **323**, 540–543.
8. CHOE H., FARZAN M., SUN Y., SULLIVAN N., ROLLINS B., PONATH P. D., WU L., MACKAY C. R., LAROSA G., NEWMAN W., GERARD N., GERARD C. and SODROSKI J. (1996): The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell, **85**, 1135–1148.
9. COLEY W. B. (1893): The treatment of malignant tumors by repeated inoculations of erisipelas: with a report of ten original cases. Am. J. Med. Sci., 105, 487–511.
10. DASCHER C. C., HIROMATSU K., NAYLOR J. W., BRAUER P. P., BROWN K. A., STOREY J. R., BEHAR S. M., KAWASAKI E. S., PORCELLI S. A., BRENNER M. B. and LECLAIR K. P. (1999): Conservation of a CD1 multigene family in the guinea pig. J. Immunol., **163**, 5478–5488.
11. FEARON D. T. (1997): Seeking wisdom in innate immunity. Nature, **388**, 323–324.
12. FEARON D. T. and LOCKSLEY R. M. (1996): The instructive

role of innate immunity in the acquired immune response. Science, **272**, 50–54.
13. FLEUREN G. J., GORTER A., KUPPEN P. J., LITVINOV S. and WARNAAR S. O. (1995): Tumor heterogeneity and immunotherapy of cancer. Immunol. Rev., **145**, 91–122.
14. GERARD C. (1998): For whom the bell tolls. Nature, **395**, 217–219.
15. HAYASHI A. (1994): Interferon γ as a marker for the effective cancer immnotherapy with BCG-cell wall skeleton. Proc. Jpn. Acad., **70**, 205–209.
16. HAYASHI A., DOI O., AZUMA I. and TOYOSHIMA K. (1998): Immuno-friendly use of BCG-cell wall skeleton remarkably improves the survival rate of various cancer patients. Proc. Jpn. Acad., **74**, 50–55.
17. HENDERSON B., POOLE S. and WILSON M. (1996): Bacterial modulins: a novel class of virulence factors which cause host tissue pathology by inducing cytokine synthesis. Microbiol. Rev. **60**, 316–341.
18. HIRSCHFELD M., KIRSCHNING C. J., SCHWANDNER R., WESCHE H., WEIS J. H., WOOTEN R. M. and WEIS J. J. (1999): Inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by Toll-like receptor 2. J. Immunol., **163**, 2382–2386.
19. JANEWAY C. A. (1989): Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb. Symp. Quant. Biol., **54**, 1–13.
20. KAWANO T., CUI J., KOEZUKA Y., TOURA I., KANEKO Y., MOTOKI K., UENO H., NAKAGAWA R., SATO H., KONDO E., KOSEKI H. and TANIGUCHI M. (1997): CD1d-restricted and TCR-mediated activation of Vα14 NKT cells by glycosylceramides. Science, **278**, 1626–1629.
21. LAMM D. L., THOR D. E., STOGDILL V. D. and RADWIN H. M. (1982): Bladder cancer immunotherapy. J. Urol., **128**, 931–935.
22. LIEN E., SELLATI T. J., YOSHIMURA A., FLO T. H., RAWADI G., FINBERG R. W., CARROLL J. D., ESPEVIK T., INGALLS R. R., RADOLF J. D. and GOLENBOCK D. T. (1999): Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. J. Biol. Chem., **274**, 33419–33425.
23. MARTIN L. H., CALABI F., LEFEBVRE F. A., BILSLAND C. A. and MILSTEIN C. (1987): Structure and expression of the human thymocyte antigens CD1a, CD1b, and CD1c. Proc. Natl. Acad. Sci. USA, **84**, 9189–9193.
24. MATSUMOTO M. and SEYA T. (1999): M161Ag is a potent cytokine inducer with complement activating function. Int. J. Molec. Med., **3**, 291–295.
25. MATSUMOTO M., SEYA T., KIKKAWA S., TSUJI S., SHIDA K., NOMURA M., KURITA-TANIGUCHI M., OHIGASHI H., YOKOUCHI H., TAKAMI H., HAYASHI A., AZUMA I., MASAOKA T., KODAMA K. and TOYOSHIMA K. (2000): IFN-γ-producing ability in blood of patients with lung cancer: production of IL-12 p40 and IL-18 in response to BCG-CWS. Int. Immunopharmacol. (in press).
26. MEANS T. K., WANG S., LIEN E., YOSHIMURA A., GOLENBOCK D. T. and FENTON M. J. (1999): Human Toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. J. Immunol., **163**, 3920–3927.
27. MEDZHITOV R. and JANEWAY C. A. JR. (1997): Innate immunity: the virtues of a nonclonal system of recognition. Cell, **91**, 295–298.
28. MEDZHITOV R., PRESTON-HURLBURT P. and JANEWAY C. A. JR. (1997): A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. Nature, **388**, 394–397.
29. MOODY D. B., REINHOLD B. B., GUY M. R., BECKMAN E. M., FREDERIQUE D. E., FURLONG S. T., YE S., REINHOLD V. N., SIELING P. A., MODLIN R. L., BESRA G. S. and PORCELLI S. A. (1997): Structural requirements for glycolipid antigen recognition by CD1b-restricted T cells. Science, **278**, 283–286.
30. MURAKAMI Y., SEYA T., KURITA M., FUKUI A., UEDA S. and NAGASAWA S. (1998): Molecular cloning of membrane cofactor protein (MCP, CD46) on B95a cell, an Epstein-Barr virus--transformed marmoset B cell line: B95a MCP is susceptible to infection by the CAM but not the Nagahata strain of the measles virus. Biochem. J., **330**, 1351–1359.
31. PRIGOZY T. I., SIELING P. A., CLEMENS D., STEWART P. L., BEHAR S. M., PORCELLI S. A., BRENNER M. B., MODLIN R. L. and KRONENBERG M. (1997): The mannose receptor delivers lipoglycan antigens to endosomes for presentation to T cells by CD1b molecules. Immunity, **6**, 187–197.
32. RESCIGNO M., GRANUCCI F., CITTERIO S., FOTI M. and RICCIARDI-CASTAGNOLI P. (1999): Coordinated events during bacteria-induced DC maturation. Immunol. Today, **20**, 200–203.
33. ROCK F. L., HARDIMAN G., TIMANS J. C., KASTELEIN R. A. and BAZAN J. F. (1998): A family of human receptors structurally related to *Drosophila* Toll. Proc. Natl. Acad. Sci. USA, **95**, 588–593.
34. ROSENBERG S. A., YANG J. C., SCHWARTZENTRUBER D. J., HWU P., MARINCOLA F. M., TOPALIAN S. L., RESTIFO N. P., DUDLEY M. E., SCHWARZ S. L., SPIESS P. J., WUNDERLICH J. R., PARKHURST M. R., KAWAKAMI Y., SEIPP C. A., EINHORN J. H. and WHITE D. E. (1998): Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat. Med., **4**, 321–327.
35. SANTORO F., KENNEDY P. E., LOCATELLI G., MALNATI M. S., BERGER E. A. and LUSSO P. (1999): CD46 is a cellular receptor for human herpesvirus 6. Cell, **99**, 817–827.
36. TAKEUCHI O., KAUFMANN A., GROTE K., KAWAI T., HOSHINO K., Morr M., MUHLRADT P. F. and AKIRA S. (2000): Preferentially the R-stereoisomer of the mycoplasmal lipopeptide macrophage-activating lipopeptide-2 activates immune cells through a Toll-like receptor 2- and MyD88-dependent signaling pathway. J. Immunol., **164**, 554–557.
37. TSUJI S., MATSUMOTO M., AZUMA I., HAYASHI A., TOYOSHIMA K. and SEYA T. (2000): Cell-wall skeleton of BCG participates in a maturation step of human dendritic cells (DC); establishment of unique mature DC from immature DC. Infect. Immun., **68**, 6883–6890.
38. WU L., GERARD N. P., WYATT R., CHOE H., PAROLIN C., RUFFING N., BORSETTI A., CARDOSO A. A., DESJARDIN E., NEWMAN W., GERARD C. and SODROSKI J. (1996): CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. Nature, **384**, 179–183.
39. YAMAMURA Y., AZUMA I., TANIYAMA T., SUGIMURA K., HIRANO F., TOKUZEN R., OKABE M., NAKAHARA W., YASUMOTO K. and OHTA M. (1976): Immunotherapy of cancer with cell wall skeleton of *Mycobacterium bovis*-bacillus Calmette Guerin: experimental and clinical results. Ann. N Y Acad. Sci., **277**, 209–227.
40. YAMAZAKI S., KOYAMA K., SOMEYA S., AZUMA I. and YAMAMURA Y. (1969): Studies on the allergenicity of various tuberculoprotein derivatives and the adjuvanticity of wax D fractions of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis., **100**, 691–698.

41. YASUMOTO K., MANABE H., YANAGAWA E., NAGANO N., UEDA H., HIROTA N., OHTA M., NOMOTO K., AZUMA I. and YAMAMURA Y. (1979): Nonspecific adjuvant immunotherapy of lung cancer with cell wall skeleton of *Mycobacterium bovis* bacillus Calmette Guerin. Cancer Res., **39**, 3262–3267.
42. ZBAR B., RIBI E. E., MEYER T. J., AZUMA I. and RAPP H. J. (1974): Immunotherapy of cancer: regression of established intradermal tumors after intralesional injection of mycobacterial cell walls attached to oil droplets. J. Natl. Cancer Inst., **52**, 1571–1577.

Received in February 2000
Accepted in May 2000