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Probiotics, dendritic cells and bladder cancer

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Probiotic foods have been available in the UK since 1996 with the arrival of the fermented milk drink (Yakult) from Japan. The presence of live bacterial ingredients (usually lactobacilli species) may confer health benefits when present in sufficient numbers. The role of probiotics in colo-rectal cancer may be related in part to the suppression of harmful colonic bacteria but other immune mechanisms are involved. Anti-cancer effects outside the colon were suggested by a Japanese report of altered rates of bladder tumour recurrence after ingestion of a particular probiotic. Dendritic cells play a central role to the general regulation of the immune response that may be modified by probiotics. The addition of probiotics to the diet may confer benefit by altering rates of bladder tumour recurrence and also alter the response to immune mechanisms involved with the application of intravesical treatments (bacille Calmette-Guérin).

What's known on the subject? and What does the study add?

The suppressor effect of probiotics on superficial bladder cancer is an observed phenomenon but the specific mechanism is poorly understood. The evidence strongly suggests natural killer (NK) cells are the anti-tumour effector cells involved and NK cell activity correlates with the observed anti-tumour effect in mice. It is also known that dendritic cells (DC) cells are responsible for the recruitment and mobilization of NK cells so therefore it may be inferred that DC cells are most likely to be the interphase point at which probiotics act. In support of this, purification of NK cells was associated with a decrease in NK cells activity.

The current use of intravesical bacille Calmette-Guérin in the management of superficial bladder cancer is based on the effect of a localised immune response. In the same way, understanding the mechanism of action of probiotics and the role of DC may potentially offer another avenue via which the immune system may be manipulated to resist bladder cancer.

KEYWORDS

probiotics, dendritic cells, bladder cancer

INTRODUCTION

Non-muscle-invasive bladder cancers represent 75–85% of all bladder cancers. The current recommendation for management is complete endoscopic resection of the tumour plus intravesical chemotherapy within 6 h. In high-risk cases, adjuvant intravesical immunotherapy with BCG may be considered. Meta-analyses have shown that intravesical chemotherapy reduces the risk of tumour recurrence while intravesical BCG reduces the risk of tumour recurrence and progression [1]. Current evidence holds that the immune system protects the host from primary cancer [2], and that dendritic cells (DCs) play a central role in the general regulation of the immune response [3]. Further studies have suggested that probiotics are capable of altering host immune function in such a way as to confer protection from infection [4] and from localised, excessive inflammatory responses [5]. We reviewed the evidence for DC activity conferring protection from recurrence of bladder tumour and if the presence of probiotics has a beneficial effect.

PROBIOTICS

Probiotics are best defined as 'a preparation of, or a product containing viable, defined micro-organisms in sufficient numbers which alter the microflora by implantation or colonization, in a compartment of the host and by that exert beneficial effects on the host' [6]. Probiotics have been shown to significantly decrease the incidence of UTI [4]. In addition, VSL #3 (a mixture of four strains of lactobacilli, three strains of bifidobacteria and one strain of *Streptococcus salivarius*) has proven effective in preventing flare-ups in chronic pouchitis in patients with ulcerative colitis [5]. Furthermore, oral ingestion of *Lactobacillus casei* may delay recurrence of bladder cancer [7]. The term probiotics covers a heterogeneous group of bacterial organisms and the mechanisms of action within the host are therefore varied with areas of overlap. These organisms exert influence by altering the micro biota, expression of cell surface molecules, secretion of bioproducts and cross talk with the host immune system. The mechanisms of action of probiotics can be divided into three broad groups [8]:

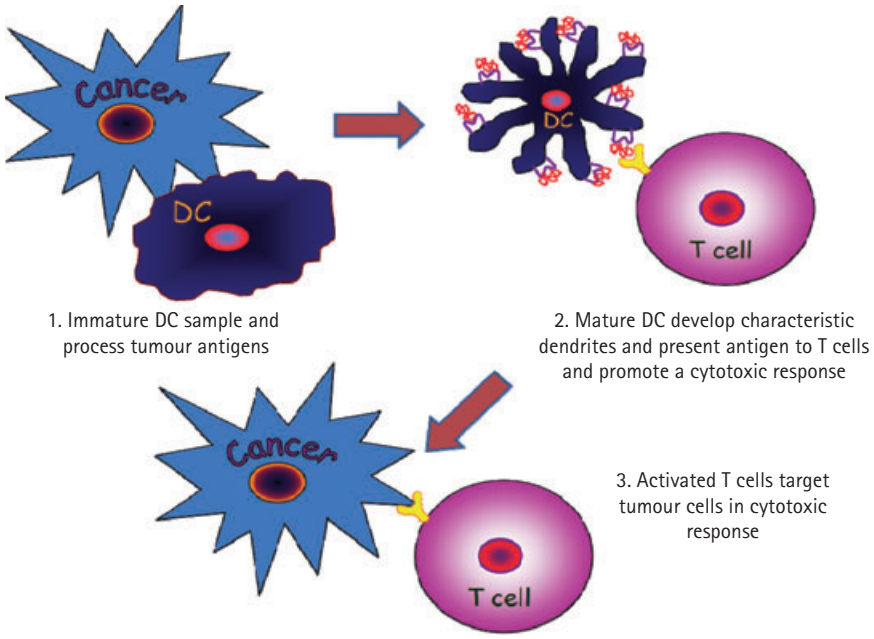
Antibacterial activity: decreasing luminal pH, secretion of bacterio-toxins, blocking bacterial adhesion to epithelial cells and inhibition of bacterial invasion.

Enhancement of mucosal barrier function: enhance barrier integrity, increase mucous production.

Immunomodulation: effect on epithelial cell cytokine secretion and survival, effect on the activity of DCs, monocytes, macrophages and lymphocytes.

Given the heterogeneous groups of live microorganisms involved and the wide

FIG. 1. DCs response to cancer.



variety in mechanism of action and thus effect, the use of probiotics therefore has to become rational and selective.

PROBIOTICS AND DCS

DCs are antigen-presenting cells and play an important role in initiation and modulation of the immune system's response to foreign antigens [3]. DCs exist in two functionally and phenotypically different states, immature and mature. Immature DCs are adept at endocytosis, after chemotactic stimuli and sampling self and foreign antigens from tissues. After stimulation by pro-inflammatory cytokines or products of microbial origin, immature DCs transform into mature DCs. Mature DCs display a reduced capability for antigen uptake but marked capacity for T-cell stimulation via presentation of antigens via Major Histocompatibility Class (MHC) molecules type I or type II, which results in activation and clonal expansion of antigen specific T-cells (Fig. 1). MHC-I bound intracellular antigens are recognised by and activate cytotoxic T-cells to directly kill target cells. MHC-II bound extracellular antigens are recognised by helper T-cells, which react by co-ordinating B-cell and monocyte activation. Depending on the cytokine milieu present at the time of T-cell priming, CD4+ T-cells are polarised towards either T_{H1} (cellular effector responses), T_{H2} (antibody responses) or regulatory T-cell responses (T_{REG}).

The T_{H1} response is characterised by increased production of interferon γ, interleukin (IL)-12, macrophage activation and differentiation of T-cells into natural killer (NK) cells. It is an aggressive pro-inflammatory response. The T_{H2} response is characterised by increased production of IL-10 which suppresses the elements of the T_{H1} reaction as well as maturation of DCs and therefore the clonal expansion of T-cells, driven by co-stimulatory molecules on mature DCs [3]. Given their central role in the immune system, it is possible within limits, to manipulate the immune system by modulating DC response with probiotics. *Lactobacilli casei shirota* (LcS) [9], *L. gasseri* and *L. Johnsonii* [10] have been shown to skew DCs towards a T_{H1} response. The probiotic cocktail mixture VSL #3 on the other hand resulted in increased IL-10, suppression of IL-12 and inhibition of DC activation and maturation typical of a T_{H2} type pro-immunoquiescence response [11,12].

L. casei is able to modulate induction of cytokines in a dose-dependent manner such that at low doses it induces release of IL-12 and interferon γ while at much higher doses it induces release of IL-10 as well. *L. casei* and other lactobacilli are able to induce DC maturation, with up-regulation of MHC-I and other cell surface molecules in a dose-dependent manner. They have variable potentiating abilities from very strong in the case of *L. casei* to the very weak for *L. reuteri*. Interestingly, *L. reuteri*, despite of being a very weak DC modulator, is able to significantly attenuate the effects of *L. casei*, resulting in a much weaker T_{H1} response [13].

A T_{H1}/T_{H2} imbalance has been suggested as the cause of inflammatory bowel disease and indeed the transmural inflammation characteristic of Crohn's disease is associated with a typical T_{H1} response [14]. This position is strengthened by VSL #3, which has proven useful in the management of inflammatory bowel disease [5]. However, the T_{H1} response is not wholly destructive and is critical to tumour surveillance mediated by both the adaptive and innate immune systems. DCs have been shown to confer a strong resistance to transfected tumours in mice. This protection was abrogated by the elimination of NK cells by anti-NK antibodies and not afforded to beige mice (NK deficient) [15].

PROBIOTICS, DCS AND BLADDER CANCER

There have been only limited reports on the anti-tumour effect of lactobacilli probiotics on bladder tumours and none that specifically investigated the role of DCs in the suppression of bladder tumours (Table 1) [7,9,15–20].

Animal studies

The first case report was the suppression of MBT-2, an experimental murine tumour by orally administered *L. casei* (LC9018) [16]. Subsequently, a *Lactobacillus rhamnosus* strain GG (LGG) [17] was reported to delay onset of carcinogenesis and tumour size in experimentally induced murine bladder tumours. In that study, which involved s.c. implantation of an established bladder tumour cell line (MB49), early introduction of LGG into the diet significantly delayed the onset of tumour development and reduced the average tumour size in mice. A quarter of tumour-bearing mice fed immediately with LGG, did not develop tumours, whilst all control and tumour-bearing mice with delayed introduction of LGG into the diet, developed tumours.

TABLE 1 The evidence for and against the possible role of probiotics and DCs in the suppression of superficial bladder cancer

For	Against
Animal studies:	
Lactobacillus casei (LC9018) suppresses experimental mouse bladder tumour [16]	
Lactobacillus rhamnosus strain GG suppresses experimental mouse bladder tumour [17]	
Dendritic cells confer protection against experimental tumors in mice but the protective effect is lost in NK deficient mice [15]	
Lactobacilli casei shirota induces increased NK cell production and activity [9]	
Lactobacillus casei protects against 3-methylcholanthrene induced tumours with associated increase in splenic NK cells activity.	
Tumour suppression is absent in NK cell-deficient mice. Separation of NK cells from splenic cells leads to reduction in cytotoxic activity [18]	
Human studies:	
Oral *Lactobacillus casei* delays recurrence of superficial bladder tumours in humans after TURBT in a small randomised trial [7].	Oral *Lactobacillus casei* is not shown to cause a significant reduction in the recurrence rate of superficial bladder tumors after TURBT in humans in a double-blind study [19].
Oral *Lactobacillus casei* augments intravesical epirubicin to delay recurrence of bladder tumour after TURBT [20].	

TURBT, transurethral resection of bladder tumour.

Analysis of immune response implied that LGG may have a mitigating effect on CD3 T-cell depletion that occurs in tumour-bearing mice while boosting the activity of CD8 and CD4 – T-cells. There was no significant difference in NK cells activity among the tumour-bearing group.

In contrast to this finding, another study reported increases in splenic NK cell activity and cytotoxicity associated with delay of superficial tumour development after LcS ingestion and intradermal injection of 3-methylcholanthrene [18]. The protective effect of LcS was absent in NK-deficient beige mice, which suggests that the anti-tumour effector cells may be NK cells. Interestingly, there was no difference in purified NK cells cytotoxicity between the LcS-fed and control groups. This may imply a splenocyte-associated potentiating agent whose effect is lost by separation of NK cells and splenocytes. In support of the effect of LcS on NK cells, Matsuzaki and Chin [9] also showed that oral administration of LcS was associated with increased activity of splenic NK cells.

Human studies

The initial human clinical trial invoved 50 patients with multiple or recurrent non-muscle-invasive bladder cancer of G1–G2 grade after transurethral resection of bladder tumour (TURBT). The patients were divided evenly into a study and control arm. A 1 g oral preparation of *L. casei*, containing $\approx 1 \times 10^{10}$ viable cells of bacterium, was administered thrice daily to the study arm and the patients were followed-up for an average of 427 days. None of the patients received chemotherapy. The results showed the 50% recurrence-free interval of the patients in the study arm was 1.8-times that of the control group [7]. On the contrary, a larger double-blinded trial in which 138 patients were enrolled, showed no significant difference in 50% recurrence-free interval between the LcS-treated and control groups [19]. However, there was a noticeable trend in increased recurrence free interval in the LcS-treated group and statistical projections indicated a significant increase in cumulative recurrence-free interval at 1 year in the LcS-treated group relative to the control group. Additionally, there was less upgrading of tumour stage in the LcS-treated group. It is noteworthy that this trial was undermined by a high discontinuation rate. The mechanism of action for the probiotic preparation was not investigated.

CONCLUSIONS

The mechanism of action by which lactobacilli appear to promote tumour resistance in humans has not been investigated but it has been implied that increased NK cell activity is likely to play a role. Separation of NK cells from splenic cells appears to inhibit enhancement of NK cell activity, which raises the question of splenocyte-associated regulatory and potentiating agents. It may be that these agents are DCs, which would suggest modification of DC activity as a focal point for the anti-tumour effect of lactobacilli. Naito *et al.* [20] have already shown that oral *L. casei* in conjunction with intravesical epirubucin resulted in significant reduction in the recurrence rate of bladder tumours after TURBT as compared with intravesical epirubicine alone. Further study is required to clarify DC-Lactobacilli interaction and it may be that rational application of probiotics eventually takes a place alongside intravesical chemotherapy and immunotherapy as adjuvant therapies in the management of non-muscle-invasive bladder tumours.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **DC**, dendritic cell; **MHC**, Major Histocompatibility Class; **IL**, interleukin; **NK**, natural killer (cells); **LcS**, Lactobacilli casei shirota; **TURBT**, transurethral resection of bladder tumour; **LGG**, Lactobacillus rhamnosus strain GG.