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Preliminary Evidence for Curative Effect of BCG on Chemically Induced Carcinoma of Mammary Gland of Female Albino Rat

Taquayya Sultana Abidi and Mohammad Tahir*
Department of Anatomy, University of Health Sciences, Lahore

Abstract.- The present study describes the possible curative effect of BCG on chemically induced breast cancer in female albino rats. Five groups, each of ten Spague Dawley adult female albino rats were administered with 100 µg 7, 12 dimethyl benz [α] anthracene (DMBA) once a week and 10 µg 12-0 tetra deconal phorbol B acetate (TPA) twice a week, for twenty and eighteen weeks, respectively, to allow development of breast cancer. Another group of ten rats was injected 0.1 ml acetone intraductally through the nipple three times a week for twenty weeks which served as control group. Four of these five groups were injected 0.5 mg Bacillus Calmette Guerin (BCG) once a week for six weeks. One out of these five groups of animals and control were sacrificed after twenty weeks whereas BCG was administered for another six weeks to the remaining four groups. These experimental animals were sacrificed one, four, eight and tweleve weeks after the last dose of BCG. The breast tissue was removed, fixed and processed for histological study. The breast tissue from animals treated with DMBA and TPA showed hyperplasia and malignant changes, whereas those sacrificed one week after BCG treatment manifested malignant and degenrating cells. Those sacrificed after four weeks showed complete absence of malignant cells, whereas the ones sacrificed after eight weeks showed recurrence of mild hyperplasia and malignant changes. The rats which were sacrificed after twelve weeks showed hyperplasia and malignant cells indicative of recurrence. It was, therefore, concluded that BCG treatment reversed the chemically induced malignancy of the breast, though recurrence was observed eight and tweleve weeks after the treatment with BCG. Further work is needed to evaluate the possibility and complete cure of the condition using BCG regime with modifications.

Key Words : Prevention, carcinogens, mammary gland carcinoma, rat.

INTRODUCTION

Carcinoma of the breast is a leading cause of cancer related mortality in female. Evidence is accumulating in recent years showing that tumours induced by carcinogens may contain tumour-specific antigens (Klein, 1966; Smith, 1968). Further, it had also been reported that the immune responsiveness of the host was depressed during latency period of carcinogenesis (Prehn, 1963; Ceglowski and Friedman, 1969). Despite the introduction of new cytotoxic drugs, improved surgical and radiotherapeutic techniques, a large proportion of carcinomas still remain incurable (Demols and Van Laethem, 2002). New targeted therapeutic strategies including immunotherapy are being explored as complementary treatment for malignancies (Mosolits *et al*., 2005). Tumour associated antigens have been targeted with BCG although it is unlikely that an active specific immunotherapy will provide a standard complementary therapeutic approach for carcinoma in the near future (Novellino *et al*., 2005).

Morales and Nickel (1986) introduced intravesical use of BCG in superficial bladder cancer and in most cases it gave better results than using it by other routes such as subcutaneous, oral and intralesional when compared with the results obtained after chemotherapy. The effect of BCG immunotherapy appeared to continue long after treatment had been stopped, the incidence of tumour reccurrence was also reduced.

BCG treatment regime is reported to produce a long term tumour regression, reduces recurrence and cancer mortality in carcinoma of urinary bladder (Lamm, 2000). The intravesical use of BCG was reported to inhibit, eradicate and prevent reccurrence of bladder malignancy. For the last three decades BCG therapy has remained the most effective local therapy for bladder cancer (Herr and Morale, 2008). Kimura (2006), reported seven out of ninteen patients of breast cancer showing partial response after oral administration of BCG.

It is well established that BCG is effective for carcinoma of urinary bladder, leprosy (Setia *et al*.,

* Corresponding author: babari1@ gmail.com
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2006). Buruli ulcer (Tanghe *et al*., 2001), diabetes type 1 (Huppmann *et al*., 2005) and colorectal carcinoma (Mosolits *et al*., 2005).

Due to high incidence of breast cancer and mortality rate associated with it, thé development of an effective vaccine may be an answer to prevent and treat the condition (Chung *et al*., 2003). Since effect of immunothrrapy had never been tried on carcinoma of breast, the present study was designed to see the effect of BCG on experimentally produced breast cancer in rats.

MATERIALS AND METHODS

This study was carried out using sixty adult healthy albino female rats of 6-8 weeks old of Sprague Dawley strain, weighing on the average 246.89±4.10 g. The animals were fed on standard rat chow and water ad libitum and were kept in controlled laboratory conditions *viz.*, 23±2°C, humidity 55±5%, dark and light cycle of 12 hours each. Ten rats served as a control and others were labelled as experimental groups. Control group of ten rats received 0.1 ml acetone, injected intraductally three times a week for twenty weeks. The remaining fifty rats received 100 µg 7, 12 dimethyl benz [a] anthracene (DMBA) once a week for twenty weeks and 10 µg 12-0 tetra deconal phorbol B acetate (TPA) twice a week for eighteen weeks. TPA injection was started 2 weeks after the begining of DMBA treatment. Twenty rats were sacrificed 24 hours after 20 weeks of the experimental period. The remaining forty rats received 0.5 mg Bacillus Calmette Guerin (BCG) (dissolved in 0.1 ml NaCl) only, once a week for a period of six weeks, and a group of ten rat each was sacrificed after one week, one, two, and three months at the end of BCG treatment.

The breast tissue was fixed it in 10% formalin for 24 hours and processed in a usual way and stained with haematoxylin and eosin. The slides were observed using (Leica DM 1000) microscope.

Gross lesion in nipple area was measured by using scale and furthur reported as mean±standard deviation. The data was analaysed using SPSS 18.0. Microscopic lesions produced in different groups of animals were compared by Fishers exact test (Swinscow, 1983). The difference was regarded statistically significant if the p value was ≤ 0.05.

RESULTS

Control

Histological sections of the breast of acetone treated rats showed ulcer of 1.06 ± 0.107 mm (n=10) in the area of the nipple (Table I). On microscopic examination, although there was no visible lesion in this group, 40% of the animals however, showed inflammatory reaction (Fig. 1).

Carcinogen- treated rats

Rats treated with DMBA and TPA for a period of twenty and eighteen weeks, respectively, showed rounded ulcer of size 2.24±0.171mm in the area of the nipple with sharp margin and granulated elevation in the center. The animals showed a marked degree of hyperplasia with mitotic figure in the cells of stratum granulosum of epidermis and fibrocytes of the dermis, extensive anaplasia and malignant changes in acini and ducts of the mammary gland. The malignant cells appeared large, hyperchromatic with large sized mitotic nuclei as compared to the normal cells. Moreover, two or three nucleoli were present in each malignant cell (Fig. 2, Table I).

BCG treated rats

One week group

The rats in this group showed shallow ulcer (1.60±0.290 mm) with inflamed margin (Table I). Microscopically six animals showed mild hyperplasia with cells having pyknotic and darkly stained nuclei in stratum granulosum of epidermis and appendages in the dermis. In 40% of the animals, most of the malignant cells, showed irregular chromatin in the nuclei which were devoid of nucleoli, indicating degenerative changes (Table I). The healing process seems to be in progress (Figs. 3, 4).

One month group

The ulcer size of 1.05±0.070 mm was detected in the area of nipple without any demarcation of margins (Table I). Microscopically

Table I.- Number of rats with lesions (cancer and hyperplasia) in control and different experimental groups having ten animals each.

Groups	No. of animals	Lesions		Size of ulcer(mm) Mean±SD
		Cancer	Hyperplasia	
Control	10	0	0	1.06±0.10
Carcinogen treated	10	6	4	2.24±0.17
Carcinogens + BCG treated				
One Week	10	4	6	1.60±0.29
Four weeks	10	0	0	1.05±0.07
Eight weeks	10	4	6	1.17±0.08
Twelve weeks	10	5	5	1.05±0.07

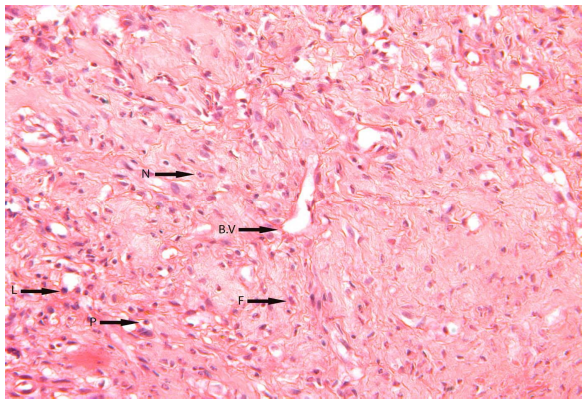

Fig. 1. Histological structure of the mammary gland from control rat treated with 0.1 ml acetone showing fibroblast (F), lymphocyte (L), plasma cells (P), neutrophil (N) and blood vessels (B.V). H&E stain, X400.

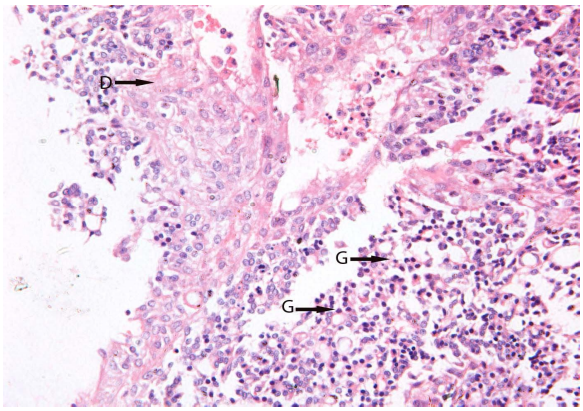

Fig. 3. Histological structure of the mammary gland of rat sacrificed one week after BCG treatment showing granulation tissue (G) and degenerated cancer cells (D). H&E stain, X400.

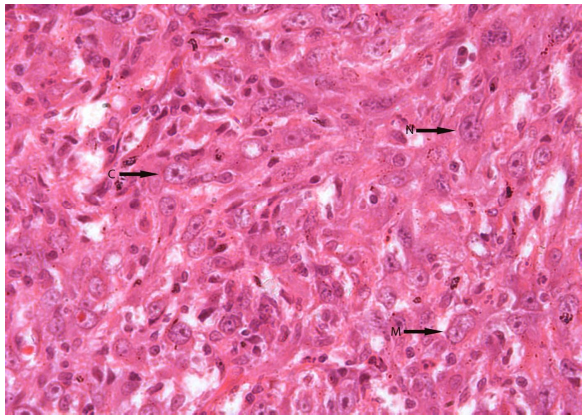

Fig. 2. Histological structure of the mammary gland of rat treated with carcinogens showing mitotic figure (M), malignant cells with large nucleus (C) and nucleoli (N). H&E stain, X400.

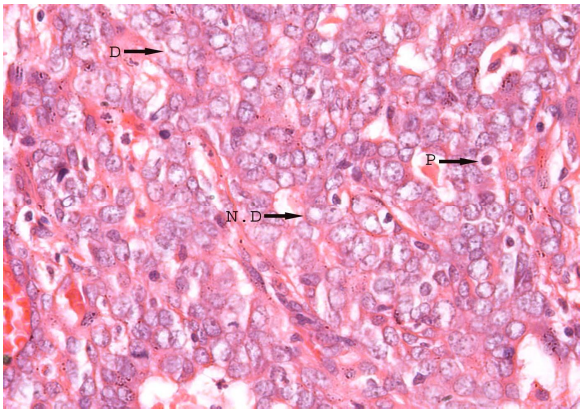

Fig. 4. Histological structure of the mammary gland of rat sacrificed one week after BCG treatment showing degenerated malignant cell (D), pyknotic nucleus (P), nuclei without nucleoli (N.D). H&E stain, X400.

the epidermis, dermis and acini of the mammary gland appeared normal without any sign of anaplasia but there was an increase in granulation tissue, fat and small blood vessels (Figs. 5, 6)

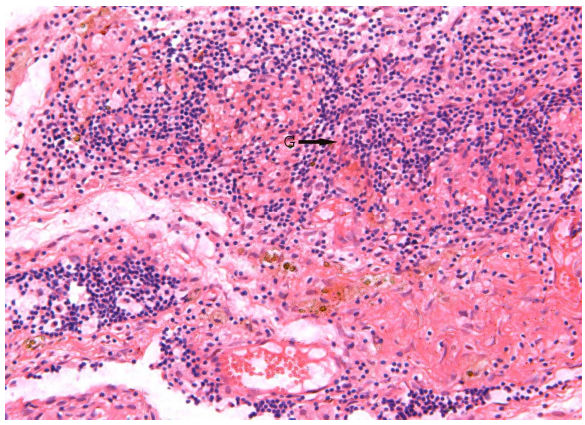

Fig. 5. Histological structure of the mammary gland of rat sacrificed four weeks after BCG treatment showing granulation tissue (G). H&E stain, X400.

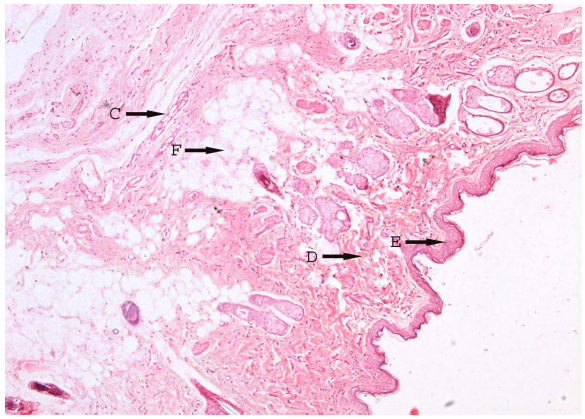

Fig. 6. Histological structure of the mammary gland of rat sacrificed four weeks after BCG treatment showing normal epidermis (E), dermis (D), fat (F) and connective tissue (C). H&E stain, X400.

Two month group

The size of ulcer was 1.170±0.082 mm in the area of nipple with inflammation in the peripheral part. Microscopically four animals showed hyperplasia, four malignant changes, and two animals were comparable to the control showing no change (Table I). There was hyperplasia of the cells in stratum granulosum of epidermis and cells contained vesicular, rounded nuclei with 2 or 3 nucleoli. Acini and ducts of the mammary gland were lined with large cuboidal epithelium cells. Moreover, malignant changes were not uniformly present in acini and ducts. The thick walled blood vessels, granulation, necrotic tissue and variable amount of fat cells were also observed (Fig. 7).

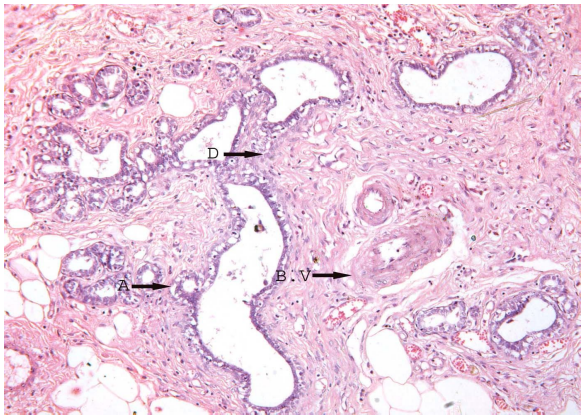

Fig. 7. Histological structure of the mammary gland of rat sacrificed eight weeks after BCG treatment showing mammary dysplasia in acini (A), connective tissue (D), and thick walled blood vessels (B.V). H&E stain, X400.

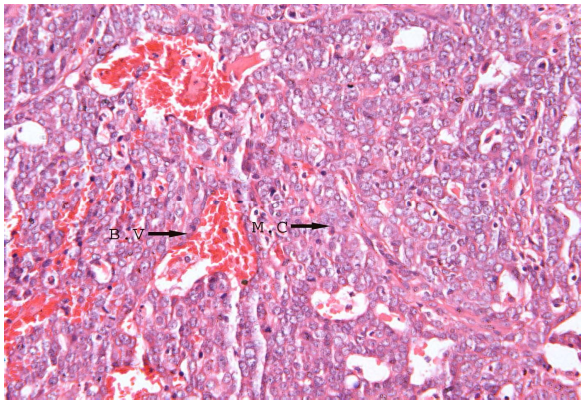

Fig. 8. Histological structure of the mammary gland of rat twelve weeks after BCG treatment showing malignant changes (M.C) and blood vessels (B.V). H&E stain, X400.

Three month group

The rats showed ulcer of 1.05±0.07 mm size and a number of granulomas. Microscopically five animals showed anaplasia and five hyperplasia (Table I). Evidence of carcinogenesis was patchy

and irregular, while acini and ducts were not uniformly affected. Variable amounts of granulation, necrotic tissue, fat and thick walled blood vessels were also observed (Fig. 8).

DISCUSSION

BCG enhances immunity against cancer (Guade, 2007). It was suggested that developing tumours exert an immunosuppressive effect on the host, while BCG exerts Immuno enhancing activity by enhancing tumoricidal activity (Gijare *et al*., 1990). Our results are comparable to those of Martinez-Pineiro and Muntanola (1977) and Morale and Nickel (1986) who administered BCG to animals by percutaneous and intralesional methods in bladder cancer.

BCG has been administered previously in bladder cancer through intravesicle and percutaneous methods (Morales and Nickel, 1986), and orally (Kimura, 2006; D´Ancona *et al*., 1991; Lamm *et al*., 1997). Later, it was demonstrated that percutaneous method of administration of BCG was no better than the intravesicle route; this was ultimately regarded as the route of choice in bladder cancer. Our results however, differ from those reported by Kimura (2006), D´Ancona *et al*. (1991) and Lamm *et al*. (1997), who found tumour regression in some cases of superficial bladder cancer.

In the current investigation majority of rats receiving 0.1 ml of acetone, injected intraductally showed no change, some animals showed minimal infiltration of inflammatory cells. This indicated that acetone acted as solvent for the carcinogens DMBA and TPA, had a minimal irritant effect. This finding is in accord with the earlier reported work, wherein acetone was used as a solvent for various agents. (ATCDR, 1995; U.S.EPA, 2003; American Chemistry Council, 2003).

The results in second group showed epithelial carcinogenesis in the mammary gland, these results were comparable to those reported by Gijare *et al*. (1990) who induced papilloma in Hamster Cheek Pouch and stomach by oral application of DMBA thrice a week for one month. Our results correlated with those reported by Rehm (1990), who induced myoepithelial mammary gland tumour by oral application of 1 mg DMBA for four weeks in mice; the tumour developed seven to eight months after stopping the treatment. The induced tumour were composed of cuboidal epithelium with microvillous border originating from lining epithelium of the duct; presence of spindle shaped cells were identified as of myoepithelial origin. Athar *et al.* (1991) induced papilloma in sencar mouse by topical application of DMBA and TPA, as in our experiment, after twenty weeks most of the papilloma progressed to malignant tumour.

Our results of experimental groups were comparable to those reported by Morales and Nickel (1986) after treatment of urinary bladder tumours by intravesicle administration of BCG; they reported that it inhibited recurrence, produced tumour regression, and cell mortality whereas the second and third month groups showed the evidence of recurrence.

In all experimental groups the granulation, necrotic and fatty tissues increased resulting in granuloma formation, which was regarded a useful defense mechanism.

Catalona *et al*. (1987) and then Pagano *et al*. (1995), improved upon the method used by Morale and Nickel (1986). They demonstrated the value of an additional second six weeks course after failure of the first course. At least 50% of the patients either with the papillary tumours, or carcinoma of urinary bladder *in situ* who failed the first course showed a response to additional course. The third therapeutic scheme is the so called maintenance scheme used by the Lamm *et al*. (1997). It is based upon the assumption that repeated treatment with BCG renews the immune response and decreases the development of recurrence.

In the light of above observations, further work is proposed, in case of breast cancer, by increasing the dose of BCG or giving a second six weeks course (Catalona *et al*., 1987; Pagano *et al*., 1995) or keeping the patient on third maintenance scheme for three years to accomplish a complete eradication of cancer (Lamm *et al*., 1997).

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REFERENCES

AMERICAN CHEMISTRY COUNCIL ACETONE VCCEP, 2003. Submission, September 10, Page 15.

ATHAR, M., AGARWAL, R., WANG, Z.Y., JENEFER, R., LIOYD, J.R., DAVID, R.B. AND MUKHTAR, M., 1991. All trans' retinoic acid protects against conversion of chemically induced and ultraviolet B-radiation induced skin papillomas to carcinomas. *Carcinogenesis*, **12**: 2325-2329.

ATSDR, September, 1995. Agency for toxic substances and disease registry.

CATALONA, W.J., HUDSON, M.A., LISSA, A., GILLEN, D.P., ANDRIOLE, G.L. AND RATLIFF, T.L., 1987. Risks and benefits of repeated course of intravesicle Bacillus Calmette Guerin therapy for superficial bladder cancer. *J. Urol*., **137**: 220-224.

CEGLOWSKI, W.S. AND FRIEDMAN, H., 1969. Murine virus leukaemogenesis relationship between susceptibility and immunodepression. *Nature*, **224**: 1318-1319.

CHUNG, M.A., LUO, Y.O., DONNELL, M., RODRIGUEZ, C., HEBER, W., SHARMA, S. AND CHANG, H.R., 2003. Development and pre clinical evaluation of a Bacillus Calmette Guerin Muci based noval breast cancer vaccine. *Cancer Res*., **63**: 1280-1287.

D´ANCONA, C.A., NETTO, N.R., CLARO, J.A. AND IKARI, O., 1991. Oral or intravesicle Bacillus Calmette Guerin immunophylaxis in bladder carcinoma. *J. Urol*., **145**: 498-501.

DEMOLS, A., AND VAN LAETHEM, J.L., 2002. Adjuvant chemotherapy for colorectal cancer. *Curr, Gastroenterol*. *Rep*., **4**: 420-426.

GIJARE, P.S., RAO, K.V. AND IBHIDE, S.V., 1990. Modulatory effects of snuff, retrinoic acid and Bcarotene on DMBA induced hamster cheek pouch carcinogenesis in relation to keratin expression, *Nutr. Cancer*, **4**: 253-259

GUADE, G., 2007. National Cancer In time June.

HERR, H.W. AND MORALE, A., 2008. History of Bacillus Calmette Guerin and bladder cancer; and immunotherapy success story. *J. Urol*., **179**:53-56.

HUPPMANN, M., BAUMGARTEN, A., ZIEGLER, A.G. AND BONIFACIO, E., 2005. Neonatal Bacillus calmette Guerin vaccination and type I diabetes. *Diabetes Care*, **28**: 1204-1206.

KIMURA, M., 2006. Effect of per orally administered BCG on recurrent breast cancer. *J. Surg. Oncol*., **20**: 65-69.

KLEIN, G., 1966. Tumour antigens. *Ann. Rev. Microbiol*., **20**: 223-252.

LAMM, D.L., 2000. Efficacy and safety of Bacillus Calmette Guerin Immunotherapy in superficial, bladder cancer, *Clin. Infect. Dis*., **31:**886-890.

LAMM, D.L., BLUMENSTEIN, B., SAROSDY, M. AND GROSSMAN, B., 1997. Significant long-term patient benefit with BCG maintenance therapy. *J. Urol*., **157**: 213-225.

MARTINEZ-PINEIRO, J.A. AND MUNTANOLA, P., 1977. Non-specific immunotherapy with BCG vaccine in bladder tumors: a preliminary report. *Eur. Urol*., **3**: 11-22.

MORALES, A. AND NICKEL, J.C., 1986. Immunotherapy of superficial bladder cancer with BCG. *World J. Urol.*, **3**: 209-214.

MOSOLITS, S., NILSSON, B. AND MELLSTEDT, H., 2005. Towards therapeutic vaccines for colorectal carcinoma: a review of clinical trials. *Expert Rev. Vaccines*, **4**: 329-350.

NOVELLINO, L., CASTELLI, C. AND PARMIANI, G.A., 2005. Listing of human tumour antigens recognized by T cells: March 2004 update. *Cancer Immunol. Immunother*., **54**: 187-207.

PAGANO, F., BASSI, P., PIAZZA, N., ABATANGELO, G., DRAGO FERRANTF., G.L. AND MILANI C., 1995. Improving the efficacy of BCG immunotherapy by dose reduction. *Eur. Urol*., **27**: 19-22.

PREHN, R.T., 1963. Function of depressed immunologic reactivity during carcinogenesis. *J. natl. Cancer Inst*., **31**: 791-805.

REHM, S., 1990. Chemically induced mammary adenomyoepithelioma and myoepithelial tumours. *Am. J. Pathol*., **136**: 575-584.

SETIA, M.S., STEINMANUS, C.S. AND RUTAERFORD, G.W., 2006. The role of BCG in prevention of leprosy-a matanalysis. *Lancet Infect. Dis*., **6**: 162-170.

SMITH, R.T., 1968. Tumor-specific immune mechanisms. *New Engl. J. Med*., **278**:1268-1275.

SWINSCOW, T.D.N. (ED.), 1983. *Statistics at Square One*. 8th ed, London; British Medical Association, pp. 54-57.

TANGHE, A., CONTENT, J., VANVOOREN, J.P., PORTAELS, F. AND HUYGEN, K., 2001. Protective efficacy of a DNA vaccine encoding antigen 85A from *Myocobacterium bovis* BCG against buruli ulcer. *Infect. Immunol*., **69**: 5403-5411.

U.S.E.P.A, 2003. *Toxicological review of acetone in support of summary information on integrated risk information system (IRIS)*, National Center for Environmental Assessment, Washington D.C. EPA/ 635/ R-03/004.

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