Ascaris antigen. The use of secretory antigens from *Toxocara* larvae seems to be very promising for a specific enzyme-immunoassay[8].

The *Dipetalonema viteae* antigen has not been used before in the E.L.I.S.A. but was of value in the I.F.A.T.[9] and in immuno-electrophoresis. However, like other antigens used for serodiagnosis of filariasis, its specificity represents a major problem (for review see Ambroise-Thomas[10]).

The high sensitivity of our hydatid fluid antigen is consistent with previous reports[11,12]. Extensive cross-reactions, particularly with filariasis sera, were striking, in agreement with the recent findings of Ambroise-Thomas[13]. Such cross-reactions have also been oberved in the I.F.A.T. using frozen sections of scolices (unpublished observations). In contrast we found the soluble antigen from scolices to be more specific but slightly less sensitive in E.L.I.S.A. (table 2). These results are in agreement with those obtained with the indirect haemagglutination test[14].

The sensitivity of our *Schistosoma mansoni* egg antigen was lower than that found by other workers[15,16] and reacted differently with sera from Europeans and from Africans. The low reactivity of some of our schistosome sera from Europeans was confirmed by tests at the Center for Disease Control (Atlanta/Georgia).

In conclusion we can say that using crude antigens and testing 1 single serum dilution, E.L.I.S.A. was inadequate for specific immunodiagnosis. For further improvements a quantitative evaluation either by photometry and/or by titration could be considered. However, preliminary studies with our antigens have shown that a higher specificity can only be achieved together with a considerable loss in sensitivity. Before purified antigens are available, a further possibility for improvement might be to test sera after absorption with different cross-reacting antigens. Initial results are promising: the extensive cross-reactivity between some filariasis and echinococcosis sera could be abolished by preabsorption with the respective antigens.

1 A. Voller, D.E. Bidwell, A. Bartlett and R. Edwards, Trans. R. Soc. trop. Med. Hyg. *71*, 431 (1977).
2 D. Bout, J.C. Dugimont, H. Farag and A. Capron, 1st Int. Symp. Immunoenzymatic Techniques. INSERM Symposium No.2, p.175. Ed. Feldmann et al. North Holland Publishing Company, Amsterdam 1976.
3 N. Weiss, D. Stürchler and F.M. Dietrich, Lancet *II*, 1233 (1978).
4 S. Tettamanti, Y. Bounameaux and V. Suter-Kopp, Schweiz. med. Wschr. *102*, 1117 (1972).
5 K.W. Walls and D.F. Palmer, Procedural guide for EIA microtitration test. US Public Health Service, Center for Disease Control, Atlanta, Georgia, unpublished, 1977.
6 G. Weiland and A. Schwarzhuber, Berl. Münch. tierärztl. Wschr. *91*, 209 (1978).
7 R.H. Cypess, M.H. Karol, J.L. Zidian, L.T. Glickman and D. Gitlin, J. infect. Dis. *135*, 633 (1977).
8 D.H. de Savigny, A. Voller and A.W. Woodruff, J. clin. Path. *32*, 284 (1979).
9 N. Weiss and A. Degrémont, Tropenmed. Parasit. *27*, 377 (1976).
10 P. Ambroise-Thomas, Acta trop. *31*, 108 (1974).
11 H. Farag, D. Bout and A. Capron, Biomedicine *23*, 276 (1975).
12 F. Sorice, S. Delia and L. Castagnari, Boll. Ist. sieroter. milan. *56*, 152 (1977).
13 P. Ambroise-Thomas, P.T. Desgeorges and D. Monget, Bull. Wld Hlth Org. *56*, 797 (1978).
14 E. Mannweiler, A. Thöle and I. Lederer, Zbl. Bakt. Hyg. I. Abt. Orig. A. *238*, 494 (1977).
15 M. McLaren, C.C. Draper, J.M. Roberts, E. Minter-Goedbloed, G.S. Lighthart, C.H. Teesdale, M.A. Amin, A.H.S. Omer, A. Bartlett and A. Voller, Ann. trop. Med. Parasit. *72*, 243 (1978).
16 V.D. Schinski, W.C. Clutter and K.D. Murrell, Am. J. trop. Med. Hyg. *25*, 824 (1976).

The effect of BCG-vaccination on vaccinia virus infections in mice

G.T. Werner

Bavarian Vaccination Centre, Am Neudeck 1, D-8000 Munich 95 (Federal Republic of Germany), 27 December 1978

Summary. Pretreatment with BCG yielded a high degree of protection against experimental vaccinia virus infections in mice. *Corynebacterium parvum* and *Aristolochia* acid were less protective; other immunostimulants were ineffective.

After application of the attenuated *Mycobacterium bovis* (BCG), an increase of nonspecific resistance against some malignant tumors has been demonstrated[1-3]. Some authors have investigated the effect of BCG against viral infections[4-7]. While studying experimental vaccinia virus infections in mice, we have compared BCG with other immunostimulants.

Material and methods. Animals: NMRI-mice, 15-25 g, were kept under conventional conditions. Immunostimulants: Levamisole (Janssen, Düsseldorf), 2.5 mg/kg s.c. once a week; Isoprinosine (Röhm Pharma, Darmstadt), 50 mg orally, daily; *Corynebacterium parvum* (Mériéux, Lyon, Lot No.R 0185), 500 µg daily s.c.; AS-3 (Madaus, Cologne), ampoules of 2 ml containing 0.461 mg *Aristolochia* acid, 0.5 ml daily s.c.; Echinacin® (Madaus, Cologne), ampoules of 2 ml containing 0.1 mg extract of *Echinacea purpurea*, 0,5 ml daily s.c.; BM 12.531 (Boehringer, Mannheim), 1-carboxamido-2-cyanaziridin[8], 50 mg/kg every 3rd day s.c.; BCG (dry vaccine Connaught, medac, Hamburg). The reconstituted vials contain 1×10^7 viable bacteria per ml.

The potency of the vaccine was tested on Lowenstein-Jensen-agar, using serial dilutions; furthermore, viable BCG were counted in the spleen cells from mice[9]. Doses of BCG: 0.1 ml once i.p. One group received a single dose of 0.5 ml orally with a gastric tube.

Virus: The neurotropic vaccinia virus strain Ma1[10] was grown on the chorio-allantoic membranes of embryonated eggs. The infectious titer came to 4.5×10^8 pox-forming units (pfu)/ml. The ID_{50} after intracerebral (i.cer.) inoculation (inoculum 0.05 ml) was $10^{2.8}$ pfu/ml according to Spaerman-Kaerber[11]. After i.p. infection it was 10^4 pfu/ml (inoculum 1.0 ml). In i. cer. infections a $100 \times ID_{50}$ was applied, in i.p. infections a $500 \times ID_{50}$.

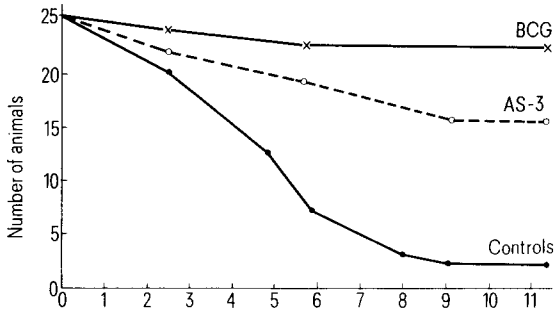
Experimental procedure. Groups of 25 mice each were given one dose of BCG either 7 or 12 days prior to the infection. *C. parvum*, BM. 12.531, Levamisole, Isoprinosine, Echinacin or AS-3 were initiated 3 days prior to the infection. In other groups the immunostimulants (except BCG) were started 6 h after the infection. All groups were monitored daily for deaths, starting with the 2nd day after infection. The experiments were terminated after 30 days. In the surviving animals either a challenge infection with $100{,}000 \times ID_{50}$ or serological investigations were performed: the pooled serum of 5 animals was examined for

neutralizing antibodies against vaccinia virus using a 50% plaque reduction in HeLa-cells.

Results and discussion. No side effects of BCG or of any other immunostimulant were observed. No drug protected the animals against i. cer. infections. Controls as well as treated animals appeared well for 2–3 days, then they became lethargic, lost appetite and developed ruffled hair. 3–4 days later they died. The mortality in all groups was 98–100%.

After i.p. infections 90% of the controls succumbed to the infection. Levamisole, Isoprinosine, Echinacin or BM 12.531 had no effect upon the course of the disease in this model. After prophylactic treatment with AS-3, 75% of the animals survived. When AS-3 was started after the infection, the survival rate was 35%. In the group, which received *C. parvum* prior to the infection, 60% of the animals survived. No effect was seen, if this drug was started after infection.

Pretreatment with BCG yielded a survival rate of 90%. There were no differences between those groups which received BCG 7 or 12 days prior to the infection (figure). The oral vaccination with BCG, however, had no effect. 20 surviving mice received an i. cer. challenge with vaccinia virus Mal 30 days after the primary infection. Wheras all aged-matched controls died, only 3 mice from the BCG group succumbed to the infection. Animals which survived the primary infection after pretreatment with BCG developed titers of neutralizing antibodies against vaccinia virus between 1:128 and 1:512. No titers were found in control animals.



Effect of BCG (×——×) and AS-3 (○——○) upon the course of the i.p. infection of NMRI-mice with vaccinia virus Mal, in comparison to untreated controls (●——●). Number of surviving animals: BCG 23/25; AS-3 18/25; controls 2/25. No further mortality after day 11 upto the end of the experiment (day 30).

Our results confirm previous reports that BCG given prior to an infection increases the resistance of animals against infections with DNS-viruses[4–7]. *C. parvum* exerts some effect if applied before the infection. The findings that AS-3 increases the survival rate, especially if it is given prior to the infection, is interesting and needs further elucidation. Why neither BCG nor any other drug protected the animals against i. cer. infection is open for discussion. Probably the course of the disease after an i. cer. infection is too rapid, and the immunostimulant might not increase the host-resistance within this short period of time. As BCG acts through activation of macrophages[12,13], it is conceivable that the macrophages do not protect against i. cer. infections, as they do not cross the blood-brain barrier. BCG treatment in patients with cancer might exert a positive effect against opportunistic infections[14], besides the increase produced in nonspecific resistance against the tumor.

1 R.C. Bast, B. Zbar and T. Borsos, New Engl. J. Med. *290*, 1413, 1458 (1974).
2 J.U. Gutterman, G. Mavligit and C.Mc Bride, Lancet *1*, 1208 (1973).
3 G. Mathé, Nat. Cancer Inst. Monogr. *39*, 107 (1973).
4 E.G. Allen and S. Mudd, Inf. Immun. *7*, 62 (1973).
5 C.J. Larson, R.N. Ushijma and R. Karim, Inf. Immun. *6*, 465 (1972).
6 H.E. Kaufmann, S. Mudd and E.D. Varnell. Invest. Ophthal. *14*, 469 (1975).
7 S.E. Starr, A.M. Visintine and A.J. Nahmias, Proc. Soc. exp. Biol. Med. *152*, 57 (1976).
8 N.A. Sher, C.C. Chaparas and J. Person, Inf. Immun. *8*, 736 (1973).
9 U. Bicker, Fortschr. Med. *96*, 661 (1978).
10 The virus was kindly provided by Prof. A. Mayr, Munich.
11 O. Bonin, Quantitativ-virologische Methodik. Thieme, Stuttgart 1973.
12 R.V. Blanden, M.J. Lefford and G.B. Mackaness, J. exp. Med. *129*, 1079 (1969).
13 C. Stiffel, D. Monton and G. Biozzi. J. reticuloendoth. Soc. *7*, 280 (1970).
14 N.A. Sher, S.D. Chaparas, L.E. Greenberg and S. Bernard, Inf. Immun. *12*, 1325 (1975).

PPD-induced blastogenesis is auto-regulated by suppressor cells generated in vitro[1]

N. Aoki and L.J. Degroot

Department of Medicine, Kinki University School of Medicine, Osaka (Japan) and Department of Medicine, University of Chicago, Chicago (Illinois, USA), 20 February 1979

Summary. Suppressor cell induction can be demonstrated during antigen specific blastogenesis by using the same methods which have shown induction of suppressor cells by Con A. Since suppressor cells are rapidly generated during antigen specific blastogenesis, they must regulate the final level of blastogenesis induced during the seven day in vitro incubation.

Regulation of a variety of immune phenomena by suppressor cells is now well established[2,3]. The inhibitory effect of suppressor cells is usually detected by mixing them with fresh cells capable of a given immune response. When the immune response of the mixed population is less than that of a control, it is supposed that suppressor cells are included in the added cells. Thus Shou et al.[4] and Hubert et al.[5] have reported human suppressor cells which are activated by preincubation with concanavalin A (Con A) and inhibit subsequent blastogenesis of syngeneic peripheral mononuclear cells (PMC) stimulated with Con A as well as other mitogens. The detection of Con A-induced suppressor activity in PMC is now a useful method for monitoring potential suppressor cell function in patients with autoimmune diseases[6,7]. However the question whether such generation of suppressor cells is a general phenomenon accompanied by a lymphoproliferative response is still uncertain. We report here that a specific antigen, purified tuberculo-