THE HELLSTRÖMS – ett intressant möte

Karl-Erik och Ingegerd Hellström delar arbetsrum och har inga tankar på att sluta forska och skriva. Efter mer än 50 år i USA pratar de fortfarande svenska med varandra.

Under min vistelse i Seattle sommaren 2018 hade jag förmånen att få träffa Ingegerd och Karl-Erik Hellström för första gången. Dessa inspirerande och framgångsrika forskare har arbetat sida vid sida under hela sin karriär. En av anledningarna att jag fascineras av deras arbete är att de tidigt insåg immunförsvarets betydelse vid cancer, något som långt ifrån alltid setts som en självklarhet.

Vi träffas i parets kontor, i en byggnad mitt emot det kända traumasjukhuset Harbour View Medical Center (förebilden till Grey´s Anatomy), där Karl-Erik och Ingegerd Hellstrom delar rum. Vi pratar svenska, efter drygt 50 år i USA talar de fortfarande svenska med varandra. Vi har inför mötet haft mailkontakt och stämt träff i entrén till Harbour View Research Building, och tjugo minuter före avtalad tid får jag ett mail med en påminnelse om vårt möte och en bifogad review-artikel om deras arbeten i olika mustumörmodeller.

Karl-Erik möter mig i lobbyn på exakt avtalad tid och berättar hur viktigt det alltid har varit för honom att hålla tider, något han lärde sig av gymnastikdirektören kapten Montan på Södra Latin. Vid försening bestraffades nämligen eleverna med en snärt av Montans florett.

Flera äldre svenska cancerforskare känner väl till paret Hellström. De träffades under sina studieår på Karolinska Institutet där intresset för cancer och immunförsvaret väcktes tidigt, och efter studierna fortsatte båda att forska hos Georg och Eva Klein. I slutet av 60-talet emigrerade familjen med två små barn till Seattle för tjänst vid University of Washington (UW). Karl-Erik berättar att de kom till Seattle med i princip tomma händer. De beviljades lån till sitt första hus och den första dammsugaren köptes på avbetalning. På den tiden anställde inte UW kvinnliga forskare och med stolthet berättar Ingegerd att detta ändrades efter deras ankomst. Paret skapade ett labb som fortfarande bedriver framgångsrik verksamhet.

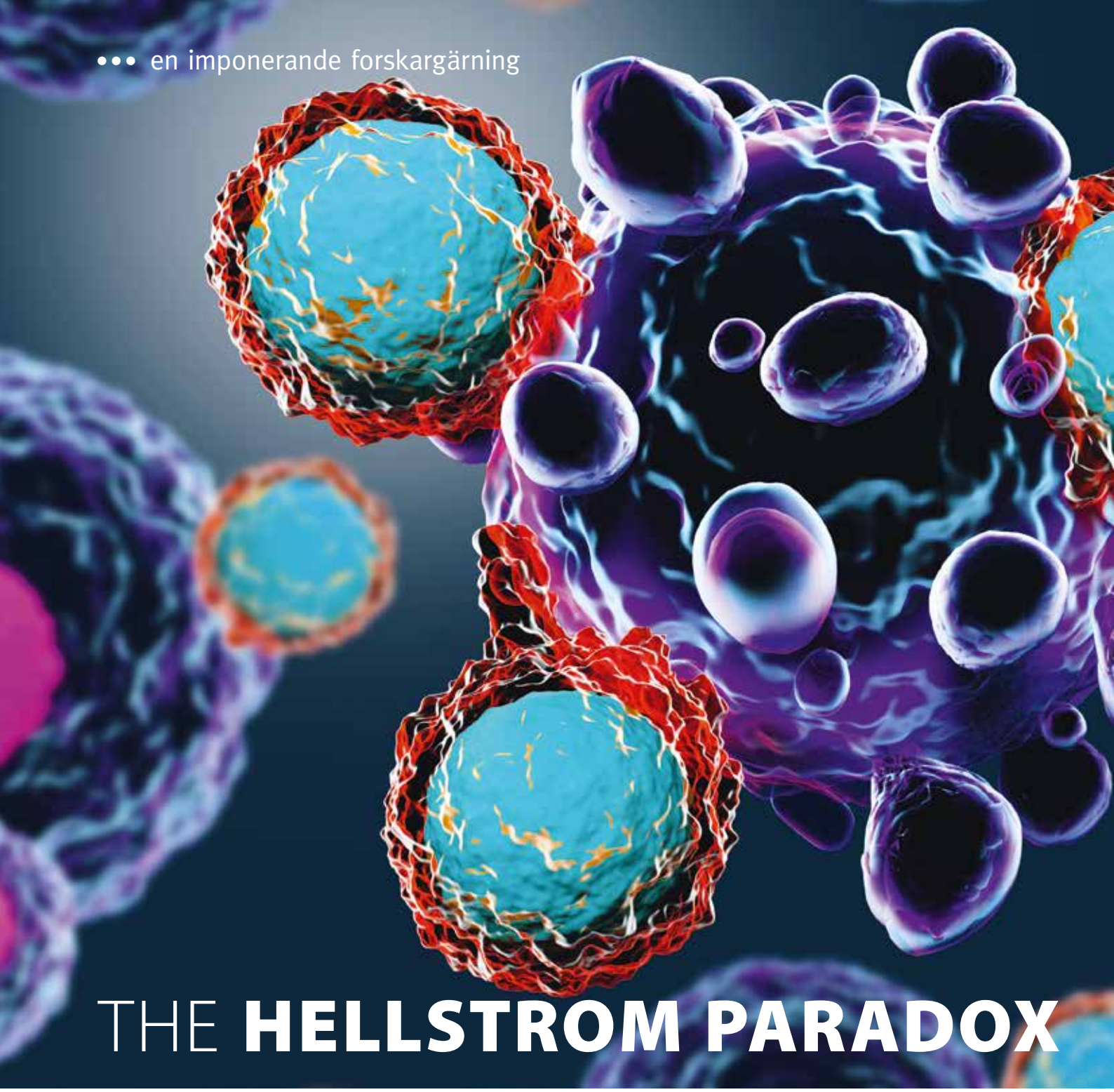
I Seattle lanserade Karl-Erik och Ingegerd ”The Hellstrom paradox” som de båda framhåller som sitt största och viktigaste bidrag till cancerforskningen.

Efter flera år på universitetet lämnade de tryggheten för att pröva på att arbeta inom ett mindre biotechbolag och senare inom läkemedelsindustrin. Efter 15 år återvände de sedan till universitetet där de båda är fortsatt aktiva.

Det var ett spännande möte med två passionerade och hängivna forskare vilkas arbete startade för mer än 50 år sedan och som beskrivs mer i detalj i deras egen text här intill. Vi har valt att publicera artikeln på engelska för att behålla deras beskrivning så autentisk som möjligt.

Det handlar om en imponerande forskning som delvis har bidragit till den utveckling vi nu ser inom immunterapin med bland annat PD1-hämmare och CAR T-celler.

HANS HÄGGLUND
PROFESSOR



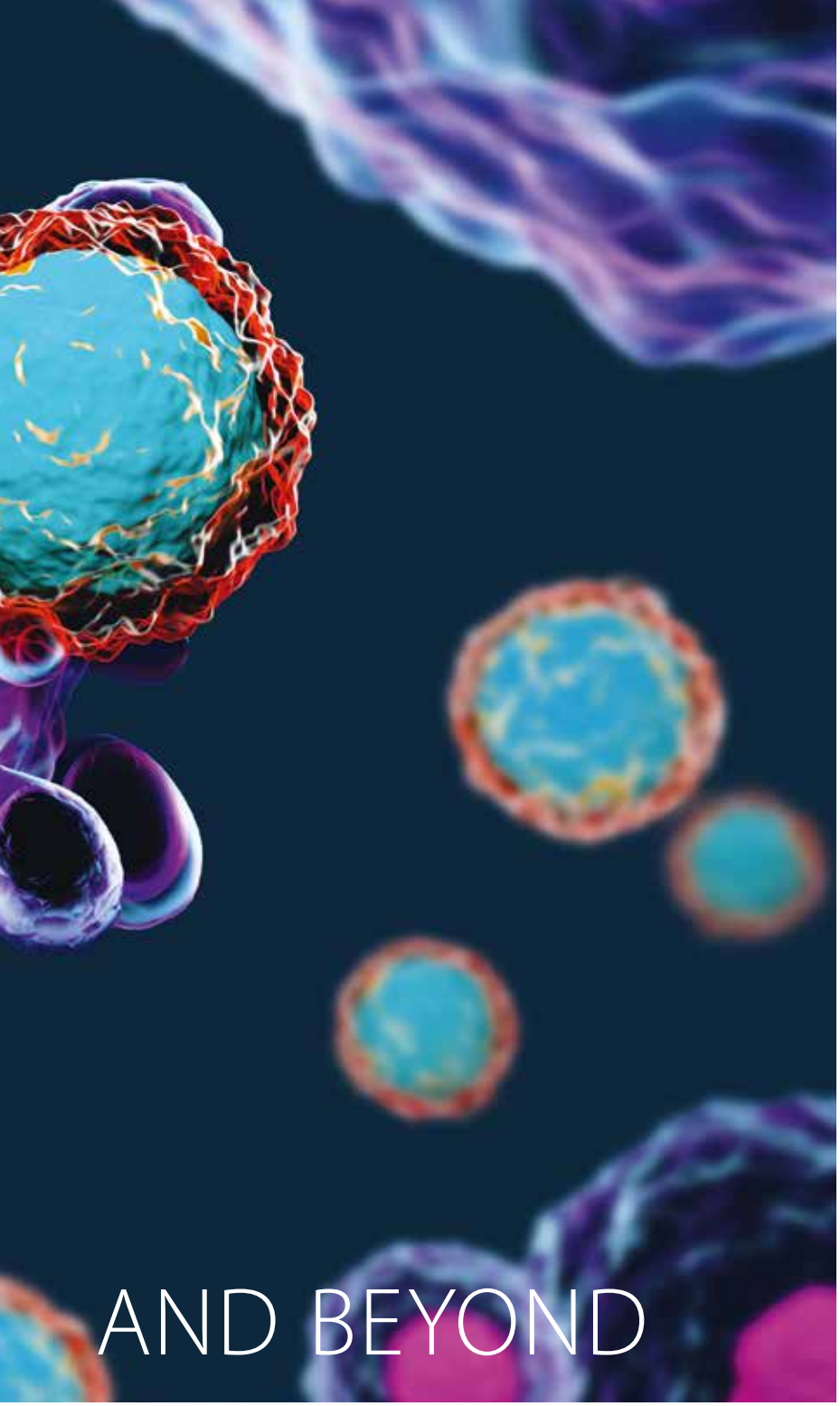
THE HELLSTROM PARADOX

Immunotherapy of cancer now attracts much attention. Cancer immunology has a long and not always flattering history, beginning more than 100 years ago when Dr William Coley[1] reported that some human cancer patients whose tumors had been injected with bacterial toxins underwent long-lasting complete remission. However, there was not much interest in this area until some 50 years later[2-6], and it was not until in the new millennium that immunotherapy for cancer began to attract much attention[7-10]. Dramatic responses have been achieved during the past decade, particularly after treatment with monoclonal antibodies (mAbs) to checkpoint inhibitors[9, 11-14], and although relatively few patients have been cured, it is likely that further developments will produce many cures by engaging the patients' immune system.

There are several reasons why immunotherapy is attractive. It can destroy tumors by cytolytic T lymphocytes[15,] NK cells[16] and macrophages[17] as well as by antibodies in conjunction with NK cells, macrophages or complement[18,] and also by TNFα and other cytokines[19] and by interference with tumor vascularization. Furthermore, the high mutability of cancer cells[20, 21] while causing problems for chemotherapeutics creates new epitopes as targets for the immune system[21].

EARLY STUDIES PAVED THE WAY

In the mid-1960s the two of us were recruited to the faculty of University of Washington in Seattle after having been among George and Eva Klein's



AND BEYOND

first doctoral students at the Karolinska. In Seattle, we started a series of experiments which showed that lymphocytes from mice, whose primary chemically induced sarcoma had been removed, could inhibit the plating efficiency of cultured cells from the same tumor[22]. The in-vitro data mirrored the in-vivo demonstration that these tumors have individually unique antigens which are targets for their rejection by immunized syngeneic mice[23]. Surprisingly, the colony formation by plated tumor cells was also inhibited when the lymphocytes came from a mouse with an established tumor.

We next performed similar experiments plating cells from short term cultures of human carcinomas together with leukocytes from the respective patients' autologous blood. The plating efficiency was inhibited and also when the lymphocytes were derived from patients who had large tumors[24-26]. Using techniques related to those we applied, these findings were soon confirmed[27-32], also at a public workshop with coded patient samples[33], but nevertheless the conclusion that human cancers can be recognized by the patients' immune system remained controversial for many years[34-36] "supported" by the belief that spontaneous tumors cannot be immunogenic[37, 38],

The surprising fact that even large tumors can be recognized as immunologically foreign has sometimes been referred to as the Hellstrom paradox[39, 40] and is related to the ability of a tumor-bearing subject to reject a small number of cells from the same tumor when transplanted outside the original tumor site[41, 42]. The paradox implies that the tumor microenvironment is highly immunosuppressive[43] and that the immunosuppression must be overcome for immunotherapy to be successful. It is noteworthy that Haydon Dong, one of the co-discoverers of PDL-1, stated that the initial goal had been to overcome the Hellstrom paradox[39].

Experiments with mouse tumors indicated that tumor antigens and antigen-antibody complexes in tumor-bearers' sera were primarily responsible for the immunosuppression by inhibiting a tumor-destructive immune response[44-50], and a high concentration of 'blocking factors' was detected at the tumor site[51]. Administering antibodies to tumor antigens could induce tumor regression in mice with Moloney virus induced sarcoma[51] and in rats with primary or transplanted polyoma virus induced tumors which supported the hypothesis that tumor antigen and complexes in antigen excess can 'block' the immune response[47, 52].

Subsequent work showed that the situation is more complex than original-

ly anticipated. For example, neoplastic cells make a variety of immunosuppressive factors[53], which include the PDL-1 and -2 ligands to the PD-1 receptor[54, 55], members of the transforming growth factor (TGFβ) family[56], IDO (indoleamine 2,3-dioxygenase)[57, 58], prostaglandin[59], AhR (blockade of IDO-kynurenine-AhR [aryl hydrocarbon receptor metabolic circuitry], NO, and others.

Furthermore, most neoplastic cells do not express the key costimulatory molecules CD80 and CD86[60] and thus display their antigens in a way that can downregulate the immune response, and engaging costimulatory signals by vaccination with tumor cells transfected to express costimulatory CD80 molecules[61, 62], or by administering agonistic anti-CD137 mAb[63] can sometimes induce tumor rejection. The power of a costimulated immune response was also demonstrated by experiments in which lymphoid cells from the blood of human patients with advanced cancer could proliferate in vitro and produce high levels of tumor necrosis factor alpha (TNFα) and IFNγ if cultured together with autologous tumor cells and stimulated by a combination of mAbs to CD28 and CD3. Importantly, they also generated tumor-destructive cytolytic T cells (CTL)[64].

TH2-TYPE INFLAMMATION PROMOTES CARCINOGENESIS

There is strong support for Virchow's postulate that inflammation promotes carcinogenesis and tumor progression[65-67]. We felt that Invasive cervical cancer (ICC) provides an ideal system to further investigate this inflammation. The etiological agent, high risk human papillomavirus (hr-HPV), is known[68], its E6 and E7 genes encode tumor specific epitopes that can be recognized by immune T lymphocytes to cause cell destruction[69-72], expression of these epitopes is intimately associated with the neoplastic transformation[73], and the lesions preceding ICC are well defined, including cervical intraepithelial neoplasia (CIN) grades 1, 2, and CIN3/carcinoma in situ (CIS)[74-78]. Chronic Th2-type inflammation is commonly seen during persistent infection with hr-HPV and promotes tumor progression[79], and immunological markers have been reported to predict regression of CIN2-3 lesions[80].

We applied immunohistochemistry to archived cervical samples to characterize the local immunological environment during the gradual progression from hr-HPV infected epithelial cells to ICC[81]. Lymphoid cells were examined for the expression of FoxP3, a marker of regulatory T cells[82, 83], CD20, a marker for B lymphocytes[84], CD138[85], a marker expressed on plasma cells and some epithelial cells, and CD32B [86], which is expressed primarily by antigen presenting cells and can downregulate an immune response after uptake of immune complexes[87]. Epithelial cells were examined for the expression of indoleamine 2,3-dioxygenase 1 (IDO1), an enzyme that induces immunosuppression[57, 88].

Already at the stage of cervical intraepithelial neoplasia (CIN1), the mucosa was infiltrated by CD20+and CD138+cells and infiltration increased in cervical intraepithelial neoplasia 3 (CIN3)/carcinoma in situ (CIS) and invasive cervical cancer (ICC), where it strongly correlated with infiltration by CD32B+ and FoxP3+ lymphocytes. GATA3+ and T-bet+ lymphoid cells were increased in ICC compared to normal, and expression in epithelial cells of the Th2 inflammation-promoting IDO1 was higher in CIN3/CIS and ICC. Thus, hr-HPV initiates a local Th2 inflammation at an early stage, involving antibody forming cells, and fosters an immunosuppressive microenvironment that aids tumor progression.

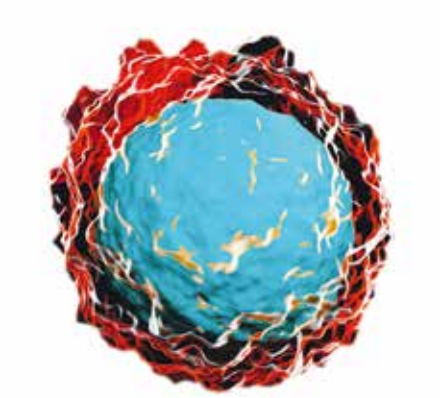
SHIFTING A TH2 TO A TH1 ANTI-TUMOR RESPONSE TO ACHIEVE TUMOR REJECTION

We found that subcutaneous transplantation of the SW1 or B16 melanoma or TC1 lung carcinoma significantly increases the number of CD19 cells in tumor-draining lymph nodes (TLN) as early as 1 day later[89]. This was in agreement with published evidence for a tumor-promoting role of B lymphocytes[90] and is reminiscent of the fact that HPV-infected human cervix uterus was infiltrated by B lymphocytes already at the CIN1 state[81]. The number of CD3 and CD8 cells concomitantly decreased[89], and there was a significantly increased number of CD11Gr-1 myeloid derived suppressor cells. RNAs were extracted from TLN 2 days after transplantation of B16 cells and evaluated by quantitative PCR. There was a statistically significant 2-fold decrease in RNA levels for IFNγ and TNFα and a 2-fold increase in the RNA level for IL4, while mice transplanted with cultured fibroblasts were not different from naïve mice according to flow cytometry or PCR. Transplanted tumor cells thus rapidly induced changes typical of a Th2 type inflammation.

We hypothesized that shifting the Th2 response to the Th1 type would promote tumor destruction. To test this hypothesis, we first performed experiments with the ID8 mouse ovarian carcinoma which shares many characteristics with human ovarian cancer[91, 92]. Mice were implanted intraperitoneally (i.p.) with ID8 cells and developed small nests of tumor cells within 10–15 days at which time they were injected with various immunomodulating mAbs i.p. or a mAb to an irrelevant control antigen. Control mice rapidly developed solid tumors and malignant ascites and were dead 24 days after tumor implantation, while injection of a combination of mAbs to CD137[63] and PD-1[93] increased survival to 55 days, and inclusion also of a mAb to CTLA4[94] further increased survival to 74 days. No survival benefit was obtained with mAbs to several other immunoregulatory (TIM-3, LAG-3) or costimulatory (OX40, GITR, CD40) molecules, as single agents and in various combinations[91, 92] and none of the tested mAbs prolonged survival when used alone.

COMBINING MONOCLONAL ANTIBODIES TO EVOKE STRONGER TH1 RESPONSE

In another experiment, mice were implanted i.p. with ID8 cells as in the therapy experiments and injected i.p. 10 days later with a combination of anti-PD1/CD137 mAbs or either mAb alone. Seven days later, the percentage, absolute numbers, and effector function markers of lymphocytes in the spleens were analyzed by flow cytometry [92]. Administration of the two mAbs resulted in a significantly increased frequency and number of



CD3+ and CD8+ T cells and decreased numbers of CD4+ and FoxP3+ Treg cells as well as of GR-1+CD11b+ myeloid-derived suppressor cells (MDSC). The levels of CD44+CD62L- effector/memory cells and of CD44+CD62L+ central memory cells were also elevated as was the number of IFNγ-producing effector CD8+ T cells, and there were decreased numbers of CD8+ T cells producing IL-10. The data thus indicated that combining the two mAbs generated a systemic Th1 type response dominated by significantly increased numbers of CD8+ effector T cells and decreased numbers of immunosuppressive cells.

Administration of anti-CD137 mAb as a single agent also increased the population of CD8+ T cells, but these cells had an 'exhausted' phenotype, failing to produce effector cytokines upon polyclonal stimulation [92]. Combined administration of anti-PD-1/CD137 mAbs blocked the upregulation of PD-1 and TIM-3 molecules on peritoneal C4 and CD8 T cells [92]. Administration of anti PD-1 mAb as a single agent had little effect on CD4+ and CD8+ T cells or on CD19+ B cells but significantly decreased the populations in spleens of immunosuppressive Treg and MDSC[92].

Tumor rejection after administration of the 3 mAb combination to mice with ID8 tumors was associated with an even stronger Th1 response with a decreased number of CD19+ cells and an increased number of CD3+, CD4+, and CD8+ cells, the last of which made large amounts of IFNγ upon polyclonal stimulation. The frequency of IFNγ+ TNFα+ CD4+ T cells in the peritoneal lavage tripled and that of CD8+ cells doubled as did the frequency of IFN-g+ TNFα+ CD4+ and CD8+ tumor-infiltrating lymphoid cells (TIL). The ratio of CD4+Foxp3- to CD4+Foxp3+ Treg cells increased as did that of CD4+Foxp3- cells to either CD8+Foxp3+ Tregs or CD11b+Gr-1+ MDSC, and the CD86+CD11c+ dendritic cells increased 5 times relative to the control.

The ID8 tumor expresses a mouse homologue of human mesothelin, which is one of the biomarkers of human ovarian carcinomas, allowing us to investigate whether the induced immune response has antigen specificity. Splenocytes from treated mice and controls were cultured in the presence of H-2Db-restricted mesothelin-specific peptide or a control HPV-E7 peptide for 3 days, after which the culture supernatants were assayed for IFNγ. Splenocytes from the treated mice, as compared to controls, secreted increased levels of IFNγ when stimulated with the mesothelin peptide as compared to the HPV peptide[91, 95]. Furthermore, splenocytes from anti-PD-1/CD137 treated mice were cytolytic to EL4 cells pulsed with mesothelin but not to cells pulsed with HPV-E7 peptide. Pre-incubation with anti-CD8 antibody suppressed the killing[92]. Other evidence that the therapy-induced response has a tumor antigen-specific component comes from studies applying the tetramer technology with the TC1 lung carcinoma[96].

These studies were expanded to include three additional models, the SW1 clone of the K1735 melanoma, the B16 melanoma, and the TC1 lung carcinoma[91]. We found anti-tumor activity including long-term tumor complete regression in all 3 models after i.p. injection of the 3 mAb combination. In most of these experiments, mice were injected with mAbs 7-10 days after subcutaneous transplantation of 106 tumor cells, at which time they had tumor nodules with a mean diameter of 4-5 mm.

INTRATUMORAL INJECTIONS FOR IMPROVED THERAPEUTIC EFFICACY

We compared the therapeutic efficacy of injecting mAbs i.p. or intratumorally (i.t.) to mice that had a subcutaneous SW1 melanoma. Given i.t., the 3 mAb combination induced CR in 65% of mice as compared to 20% CR in mice injected i.p.[89]. The i.t. injection had the best therapeutic activity both against an injected and an untreated tumor in the same mouse[89, 91].

To investigate the role of CD4+, CD8+, and NK cells in the therapy-induced antitumor response, mice injected with the 3 mAb combination were injected i.p. with mAbs to the respective cell populations. In the ID8 model, depletion of CD4+ or CD8+ cells abrogated the antitumor effect, while depletion of NK cells had a marginal effect. In the SW1 model, CD4+ cells were needed for therapeutic efficacy while the differences were not statistically significant for CD8 and NK cells.

TLNs and spleens were harvested from mice whose B16 melanoma either had completely regressed ("regressors") or were growing progressively ("progressors") after three i.t. administrations of the 3 mAb combination. Regressors had significantly more CD44CD62L- effector memory cells than progressors, which is consistent with shifting of the tumor microenvironment from an immunosuppressive Th2 to an immunostimulatory Th1 type[91]. Further evidence that a therapeutic response was caused by such a shift came from experiments in which the 3 mAb combination was injected once i.t. into SW1 tumors of 4–6 mm diameter and the mice euthanized 7 days later. TLN and spleens were enlarged as compared to controls and there was a dramatic increase in the number of CD3, CD8 and CD4 cells and of CD11CD86 mature DC in both TLN and spleen. The number of cells expressing CD137 was also increased, while the populations of cells expressing PD-1 or CTLA-4 was unaffected. The treated mice had significantly increased numbers of CD4 and CD8 cells producing IFNγ and TNFα and significantly increased numbers of CD11CD86+ mature DCs. Infiltration by CD19 cells was decreased compared to controls.

RESPONDERS AND NONRESPONDERS

In another series of experiments, we compared tumor-infiltrating lymphoid (TIL) cells from B16 melanomas which had either started to regress ('responders') or were growing progressively ('nonresponders') when the mice were

euthanized 7 days after the second i.t. injection of the 3 mAb combination. Responding tumors contained much fewer CD19 cells and more CD3, CD4, and CD8 cells of which 90% were CD44CD62L- effector memory cells as compared to nonresponders and controls which were similar. Moreover, responding tumors had 6-fold higher mRNA levels of IFNγ and TNFα, 2-fold higher mRNA levels of perforin and a 15-fold increase of granzyme B mRNA and they had significantly decreased numbers of CD19+ cells, increased IFNγ and TNFα producing CD4+ and CD8+ T cells and mature CD86+ DC and increased ratios of effector CD4+ and CD8+ T cells to CD4+Foxp3+ Treg and to CD11b+Gr-1+ MDSC.

According to PCR array analysis of TLN from mice with SW1 tumors, the 3 mAb combination upregulated transcription of the Th1-related molecules IFNγ, Stat4, and TBX21 and downregulated transcription of the Th2 molecules IL-4, Stat6, and GATA3[91], which is consistent with an increased percentage of IFNγ producing T cells according to intracellular cytokine staining. There was also an increase of the Th1-associated chemokine/receptor CXCL9-11/CXCR3 and a decrease of the B-cell chemotactic chemokine/receptor CXCL13/CXCR5, which is concordant with an increase of CD4+ and CD8+ cells and decrease of B cells in TLN. Upregulation of CXCL9 and of CXCL10 and CXCL11 was confirmed by qPCR in both tumors and TLN from treated mice demonstrating a shift of the immune response toward Th1 type by the 3 mAb treatment[91].

COUNTERACTING TUMOR-PROMOTING ROLE OF B CELLS

As in the ID8 ovarian cancer model, regression of SW1, B16 and TC1 was associated with a significantly decreased number of CD19 cells in tumors, TLN and spleen [89, 91], probably because of the anti-B cell activity of agonistic mAbs to CD137[97], and we hypothesized that the therapeutic efficacy could be improved by also including a mAb to CD19. To test this hypothesis, mice with a 4–5 mm SW1 tumor on the right side of the back and a 2–3 mm such tumor on the left (to investigate whether there was a systemic effect) were injected with either the 3 mAb combination or anti-CD137/PD-1/CTLA4/CD19 ("the 4 mAb combination") into the right tumor. Treatment with the 4 mAb combination produced an overall survival of 59 days compared to 35 days with the 3 mAb combination and 18 days in controls, and 60% of mice receiving the 4 mAb combination were alive, tumor free, 100 days after the first treatment. Subsequent studies showed that the 4 mAb combination also was more effective in the B16 and TC1 models[89, 98].

Experiments were also performed with mice whose s.c. SW1 melanoma had a mean diameter around 8-9 mm at the onset of therapy[89] and showed that the 3 and 4 mAb combinations produced 10 % and 60% CR respectively. In mice with B16 tumors of approximately the same size, the 3 mAb combination only prolonged survival from 10 to 30 days while the 4-mAb combination induced long-lasting CR (and probably cure) in 50% of the mice. However, it was much easier to induce regression of small tumors which is reflected by a greater difficulty in inducing a shift from a Th2 to a Th1 response as tumors are larger[96].

Mice whose SW1 tumors had undergone CR were transplanted s.c. with SW1 cells 140 days after the first treatment, as were age-matched naïve controls. The SW1 cells were rejected by 80% of regressor mice, which remained tumor free when the experiment was terminated after more than 200 days[98], i.e. treatment had induced a long-term response.

We then investigated the immunological profiles of mice whose B16 tumors were injected 7 days previously with either the 3 or 4 mAb combinations, to compare the two combinations, or with individual mAbs[96]. There were more CD3, CD4, CD8, and CD80CD11c cells and fewer CD19 cells in TLN from mice whose tumors were injected with the 4 mAb combination and this combination also induced more CD8 cells in the spleen, a higher frequency of T effector to Treg cells in tumors. Furthermore, it more effectively increased the number of TIL which expressed CD137 and CD86 and the number of CD44CD62L central memory CD8 T cells as compared to the 3mAb combination, and better increased the shift from a Th1 to a Th2 response with an increased Tbx21/Gata3 ratio, higher mRNA expression of IFNγ, and lower mRNA expression of IL4[96].

The therapy-induced Th1 response declined within weeks after mAb administration, which probably explains why approximately 20% of macroscopically regressed tumors relapsed within 2 months following treatment. The relapsed tumor cells retained full sensitivity to the original treatment protocol, while TIL, TLN, and spleen of relapsing mice had the characteristics of a Th2 type inflammation, i.e., the relapses were caused by failure of the immune system to retain a tumor-destructive Th1 tumor environment[96].

SYNERGISTIC EFFECT COMBINING IMMUNOMODULATORY MABS WITH CISPLATIN

Stimulated by the report that anti-CD137 mAb synergizes with cisplatin to induce regression in a mouse colon cancer model[99], Wei et al. investigated whether cisplatin synergizes with a combination of immunomodulatory mAbs[92] to obtain preclinical data which could be translated to a clinical protocol for such patients. The approach was encouraged by the demonstration that i.p. injection of mAbs to CD137 plus PD-1 doubled survival of mice with the ID8 ovarian carcinoma[91].

Ten days after implanting C57BL mice with ID8 cells the mice were injected i.p. with 10 mg/kg cisplatin followed by 2 doses of anti-PD-1/CD137 mAbs at a 4-day interval. Controls received the same protein dose of an irrelevant mAb. Confirming previous data, a doubled survival time was observed with the mAb combination, but there were no long-term survivors, and no survivors among mice only given the drug. However, 8 of 10 mice receiving both cisplatin and the 2 mAb combination survived healthy and tumor free when the experiment was terminated after a 100 days observation period[92, 100]. The surviving mice rejected challenge with ID8 cells while cells from the syngeneic but antigenically

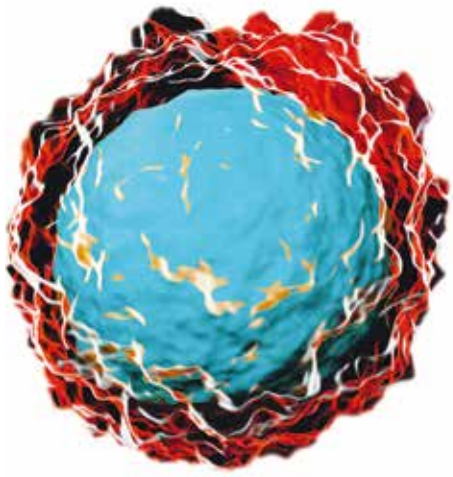
different TC1 lung carcinoma grew progressively.

Treated mice had a significantly increased total number of CD8+ T cells in the peritoneal lavage and spleens and these cells were functional, as demonstrated by antigen-specific cytolytic activity and IFNγ production, and by the demonstration that removal of CD8+ T cells abrogated the anti-tumor effect[92, 96]. Administration of anti-CD137 mAb as a single agent similarly increased CD8+ T cells but these had no functional activity due to upregulation of co-inhibitory PD-1 and TIM-3. Combined CTLA4 blockade and CD137 triggering was not therapeutically effective either, indicating that PD-1 mediated negative signaling plays a more important role than immunosuppression via CTLA-4 in the evasion of ID8 cells from immunological control.

In another set of experiments, mice with s.c. TC1 tumors of 4–5 mm diameter were injected i.p. with cisplatin, 10 mg/kg, immediately followed by various mAb combinations given i.t. and repeated 2 times weekly. Combination of mAbs to CTLA4 plus PD-1 with cisplatin induced long term CR in most of the mice[96]. The ability of cisplatin to counteract the immunosuppressive tumor microenvironment[101], including a population of tolerogenic and IDO positive MDSC[102] probably contributed to the beneficial effects[96]. We speculate that the therapeutic efficacy can be further improved by drugs that counteract the induction of IDO by IFNγ, whose level is increased by the immunomodulating mAbs [103], and that therapeutic vaccination, radiotherapy, or various other drugs can synergize with mAbs to checkpoint inhibitors. Combination of radiotherapy with immunomodulatory mAbs has already been shown to act synergistically[104–106].

Translation of these findings to the clinic should be considered. Although no toxicity beyond occasional hair loss was observed in treated mice, caution must be exercised particularly since anti-CD137 agonistic antibodies have been observed to cause toxicity in both preclinical models[107] and clinical trials [108]. Restricting the biodistribution of the mAbs to the peritoneal cavity, e.g., by entrapment in nanoparticles, may provide a targeted approach to elicit effective antitumor immunity while minimizing systemic toxicity[109].

Abstract. Carcinogenesis and tumor progression are associated with Th2 mechanisms and regression with a shift



••• en imponerande forskargärning

to a Th1 response. This shift is more difficult to achieve with larger tumors, and one is reminded of the fact that rejection of transplanted tumor cells by an immunized mouse is commonly overcome by just increasing the number of transplanted cells with a couple of logs, i.e. tumor-related immunosuppression is very effective. Importantly, the Th2 to Th1 shift can be facilitated by combining immunomodulatory mAbs with cisplatin which, in addition to killing tumor cells, also impacts the immune system[43, 110], and a combination of the clinically approved anti-PD1, anti-CTLA4 and cisplatin induces a Th1 type response with CR when neither the drug nor the mAbs were effective[96] by themselves. It will be important to learn whether combination of immunomodulatory mAbs with inhibitors of IDO, AHR, PDL-1 or -2, TGFβ and/or other immunosuppressive molecules, drugs or mAbs will further increase the efficacy. The most impressive clinical responses to immunomodulating mAbs have been seen with mAbs to PD-1, PD-L1 and CTLA4, i.e., with mAbs that target tumor-related immunosuppression. Agonistic mAbs to costimulatory receptors such as CD28 have been less therapeutically beneficial and display much toxicity.

In our studies, i.t. injection of immunomodulatory mAbs was superior to systemic delivery to obtain both a local and a systemic effect. For i.t. delivery, enclosing the injected material in nanoparticles[109] may prolong its stay at the tumor site, and for targeting to tumors the surface of nanoparticles may be 'decorated' by anti-tumor mAbs. If small molecules can be identified with similar functions as the respective immunomodulating mAb, it may be possible to make immunoconjugates for systemic delivery with tumor targeting by coupling to an anti-tumor mAb.

Relapse of treated TC1 carcinoma was seen in about 20% of cases[96]. Importantly relapse was associated with a shift from a tumor-inhibitory Th1 response to one of the Th2 type while the relapsing tumor cells retained their sensitivity to treatment with immunomodulatory mAbs. It will be important to investigate whether one can decrease the frequency of relapses by prolonging the treatment with mAb and/or drug or by combination it with therapeutic vaccination or adoptively transferred T cells.

Major efforts should be devoted to investigating side-effects. Although toxicity has not been a large problem in our studies, a concern is that immunomodulatory mAbs can cause autoimmune reactions[111–113], and there is a need to detect and treat autoimmune side-effects early. There is a need for highly effective mAbs or other therapeutic agents to shift the tumor-promoting Th2 response to one of the Th1 type.

A review article covering our work in this area with more detailed presentation is being published[114].

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