PIBF: The Double Edged Sword. Pregnancy and Tumor

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Problem
The role of progesterone-dependent immunomodulation in the maintenance of normal pregnancy.

Methods
In vitro and *in vivo* data on the effect that progesterone and its mediator progesterone-induced blocking factor (PIBF) exert on the immune functions of pregnant women are reviewed, together with clinical findings.

Results
Activated pregnancy lymphocytes express progesterone receptors, which enable progesterone to induce a protein called PIBF. PIBF increases Th2 type cytokine production by signaling via a novel type of IL-4 receptor and activating the Jak/STAT pathway. PIBF inhibits phosholipase A2, thus reduces prostaglandin synthesis. PIBF inhibits perforin release in human decidual lymphocytes and reduces the deleterious effect of high NK activity on murine pregnancy. PIBF production is a characteristic feature of normal human pregnancy, and its concentration is reduced in threatened pregnancies. PIBF mRNA and protein are expressed in a variety of malignant tumors. Inhibition of PIBF synthesis increases survival rates of leukemic mice.

Conclusion
Progesterone-induced blocking factor is produced by pregnancy lymphocytes and also by malignant tumors. The PIBF-induced Th2-dominant immune response is favorable during pregnancy but might facilitate tumor growth by suppressing local antitumor immune responses.

Introduction

In humans, progesterone is crucial for establishing and maintaining pregnancy. The underlying mechanism is rather complex and includes – among others – an interaction between progesterone and the immune system. High concentrations of progesterone have been shown to prolong the graft survival[1,2] and to affect various phases of the immune response *in vitro*.[3–5]

At the materno-fetal interface, the concentration of locally synthesized progesterone reaches 3–10 μg/mg of placental tissue. The serum concentrations in pregnant mice and humans are however much too low (100–500 nM) to support the concept of generalized immunosuppression.[6,7]

The immuno-modulating effect of progesterone is determined – on the one hand by availability of the hormone, and on the other hand by progesterone sensitivity of the lymphocytes.

While relatively low (100–400 nM) progesterone concentrations are sufficient to inhibit peripheral NK activity in healthy pregnant women, 100-fold higher concentrations are required for the same effect in non-pregnant individuals.[8] The effect of progesterone on pregnancy lymphocytes is inhibited by progesterone receptor block,[9] RU486 treatment of peripheral pregnancy lymphocytes significantly

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augments NK cell cytolytic activity *in vitro*, and this can be reversed by treatment of NK cells with progesterone.[10] This, together with the high progesterone sensitivity of pregnancy lymphocytes suggests a receptor-mediated action of progesterone in these cells.

Lymphocyte progesterone receptors

The presence of specific progesterone receptors (PR) in peripheral pregnancy, but not non-pregnancy lymphocytes, was demonstrated for the first time by our group.[11] The majority of progesterone receptor-positive pregnancy lymphocytes were γ/δ TCR+[12,13] and/or CD8+.[11] During normal pregnancy, peripheral blood progesterone receptor-positive lymphocytes increase in number. Spontaneous labor, miscarriage or pre-term delivery are characterized by the absence or at least marked reduction in the numbers of PR+ lymphocytes,[14] suggesting that the loss of lymphocyte PRs might lead to the termination of pregnancy.

The expression of lymphocyte PRs is regulated in a hormone-independent manner. Activation of the lymphocytes *in vitro*[11] and *in vivo*[15] results in PR expression. Furthermore, lymphocyte immunotherapy for recurrent miscarriage has been shown to induce lymphocyte PRs and these to be related to the success or failure of gestation.[16]

These data suggest that the induction of lymphocyte PR-s is activation-dependent and that the emergence of these receptors during gestation is not because of the altered hormonal environment, rather to immunological recognition of pregnancy.

There is ample evidence now that maternal recognition of fetal antigens is important for a normal pregnancy outcome.[17–24] The fetus itself does not come into direct contact with maternal tissue. It is the embryonic trophoblast, which forms the interface between the maternal and fetal compartments, allowing recognition of fetal antigens as well as effector mechanisms to take place. On the transcriptional level, HLA-A, HLA-B, HLA-C, HLA-E and HLA-G are present on individual trophoblast populations, but only HLA-C HLA-G and HLA-E are translated to proteins.[25,26] Antigen recognition by CD8+ T cells is polymorphic MHC restricted; therefore, it is unlikely that α/β T cells are able to recognize trophoblast-presented antigens.

Close to 70% of decidual T cells express the γ/δ T cell receptor,[27,28] and most of these cells are activated.[29,30] γ/δ T cells recognize a limited group of ligands.[31–33] They react with unprocessed foreign antigens in a MHC non-restricted manner, and also with non-polymorphic Class I or Class I like molecules.[34,35]

Therefore, this population is a likely candidate to recognize trophoblast-presented fetal antigens.

In peripheral blood of pregnant women, there is an increased presence of γ/δ TCR-positive lymphocytes. More than 90% of these cells are activated, express PRs[12] and react with non-classical HLA antigens.[36]

De León-Nava et al.[37] demonstrated PRs in lymphocytes of gonadectomized female, but not of male mice. Butts et al.[38] reported that mature bone marrow-derived dendritic cells from female rats were more sensitive to progesterone than those from males. Recently, Dosiou et al.[39] described a new immunomodulatory pathway by progesterone, through G-protein-coupled membrane PRs in lymphocytes.

Purified decidual NK cells do not express PRs,[40] while both classical PR-A and B have been demonstrated in KIR(+), but not in CD56(bright) KIR(−) peripheral blood NK cells.[41] It is suggested that the latter might represent the peripheral blood NK precursors selectively recruited into the endometrium where they differentiate to become the uterine NK cells.

Progesterone-induced blocking factor

Steroid hormone action includes a structural alteration of the receptor upon hormone exposure, which in turn enables DNA binding[42] and the induction of genes leading to protein synthesis.

Several effects of progesterone are mediated by a lymphocyte-derived protein, called progesterone-induced blocking factor (PIBF).[43] The PIBF1 cDNA encodes a protein of 757 amino acid residues with a 90-kDa predicted molecular mass, which shows no significant amino acid sequence homology with any known protein.[44] The full-length PIBF is associated with the nucleus, whereas secretion of shorter splice variants is induced by activation of the cell.[45]

Progesterone-induced blocking factor affects cytokine synthesis, NK activity as well as arachidonic acid (AA) metabolism (Fig. 1).

The effect of PIBF on cytokine production

In humans, there is a well-established relationship between the peripheral cytokine pattern and the

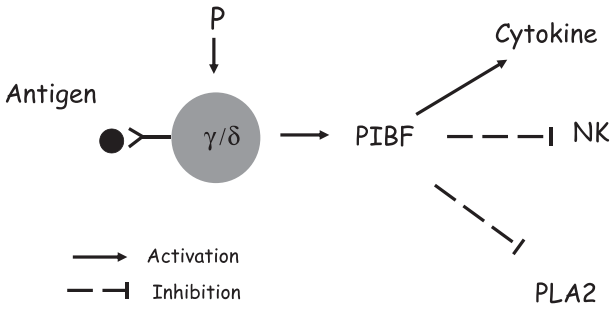

Fig. 1 The induction and effects of progesterone-induced blocking factor. PLA2 phospholipase A2.

outcome of pregnancy. It has been suggested that significantly Thl-dominant cytokine production might represent the underlying phenomenon of reproductive failure.[46,47] Activated peripheral blood mononuclear cells of women with recurrent miscarriage have been shown to produce predominantly IL-2, TNFα and interferon.[48,49]

Rezaei and Dabbagh[50] reported that sera from 92 women with three or more previous miscarriages contained significantly higher concentrations of TNFα and β as well as IL-2, than those of healthy pregnant women. In another study, mitogen-activated peripheral lymphocytes obtained from recurrent aborters at the time of miscarriage and even 3 months later produced more IL-2 than those from women without a history of miscarriage.[51] Wilson et al.[52] showed that among 115 pregnant women with a history of recurrent miscarriage, those who miscarried had increased serum levels of the Th1-associated cytokines IFN-γ, IL-12 and IL-18 compared with healthy pregnant women, implying that cytokine measurement might have a value in predicting miscarriage.

In contrast to the above-mentioned studies, Bates et al.[53] reported increased IL-4 and IL-10 production and decreased IFNγ and TNFα production in women with recurrent miscarriage patients compared to normal pregnancy.

A recent prospective clinical trial revealed that progestogen-induced PIBF production down-regulates peripheral Thl cytokines and stimulates Th2 cytokines in women with recurrent miscarriage.[54] Both human[55,56] and animal[57] studies suggest that inducing PIBF production could be the indirect mechanism by which progestagens improve pregnancy outcome.

Progesterone-induced blocking factor induces increased production of Th2 type cytokines[58] by signaling via the Jak/STAT pathway.[59] PIBF activates STAT6 and inhibits STAT4 phosphorylation, furthermore, silencing of STAT6 by siRNA interferes with cytokine effects of PIBF.[59] Because the activation of the STAT6 pathway depends on the ligation of IL-4R,[60] for activating STAT6, PIBF needs to interact with IL-4R. Indeed, the STAT6 activating effect of PIBF is lost if the IL-4R α-chain is blocked by a specific antibody. Blocking of the IL-13R (which consists of an IL-13 binding IL-13R α-chain[61,62] and IL-4Rα[63]) does not exert a similar effect, suggesting that the IL-4R α-chain is needed for STAT6 activation by PIBF. PIBF does not directly bind to IL-4Rα, nor does anti-IL-4Rα antibody treatment prevent PIBF from binding to its own receptor. IL-4R block inhibited PIBF-induced Jak1 phosphorylation, whereas the blocking of IL-13R did not. Because Jak1 is associated with IL-4Rα, this implies that both the positive and negative effects of PIBF depend on the IL-4Ra-chain. With confocal microscopy, we demonstrated co-localization and ligand-induced co-capping of IL-4Rα and the PIBF receptor. Therefore, the hypothesis was put forward that, following ligation, the PIBF receptor might form a heterodimer with the α-chain of the IL-4R, allowing PIBF to activate the STAT6 pathway.

These findings raise the question, why IL-4Rα is needed for PIBF signaling. A plausible explanation would be that the PIBF receptor itself does not possess an intracellular domain; therefore, it uses that of IL-4Rα. Several proteins are anchored to membranes via a post-translational lipid modification, the GPI anchor. Ligation of these proteins by Abs results in signal transduction, despite the fact that these molecules have no transmembrane or intracellular domains. They acquire signaling capacity by associating with putative transmembrane proteins that can signal via conventional mechanisms.[64]

Testing the hypothesis that the PIBF receptor was a GPI-anchored protein, we digested the anchoring region with PI-PLC. After this treatment, IL-4 was still able to activate STAT6, but PIBF failed to do so, suggesting that a GPI-anchored protein was involved in PIBF signaling. GPI deficiency causes female infertility in mice,[65] but to date the protein needed for maintaining pregnancy has not been identified. PIBF deficiency ablates murine pregnancy, suggesting that the PIBF receptor might be considered a candidate.

The concept that both IL-4Rα and PIBF receptor are required for PIBF signaling is supported by the following: (1) anti-IL-4Rα does not prevent binding of PIBF to its receptor, suggesting that PIBF receptor and IL-4Rα are separate entities; (2) digesting the GPI anchor abolishes PIBF-driven signaling, thus a GPI-anchored protein is required for PIBF signaling; and (3) anti-IL-4Rα Ab inhibits PIBF-induced STAT6 phosphorylation in intact cells showing that PIBF cannot signal via its own receptor without the involvement of IL-4Rα. Taken together, the GPI-anchored PIBF receptor is required, but not sufficient, for PIBF signaling.

These data suggest the existence of a novel IL-4R, which consists of the engaged PIBF receptor and the α chain of the IL-4R.

By increasing the production of Th2-type cytokines, PIBF might stimulate antibody synthesis by B cells. A population of antibodies – owing to the presence of a mannose-rich oligosaccharide residue on one of the Fab arms of the molecule – possesses an asymmetric structure.[66] Nearly 10% of serum IgGs are *N*-glycosylated in the variable region.[67] The presence of an additional glycan may alter the conformation of the antigen binding site, potentially leading to beneficial or harmful effects on the host.[67] By acting on glycosylation of IgG PIBF favors the production of asymmetric IgG.[68] In pregnant women, there is a positive relationship between asymmetric antibody content of the sera and PIBF expression on lymphocytes.[68] In PIBF-deficient pregnant mice, the percentage of asymmetric antibodies is lower than in controls.[68]

The effect of PIBF on arachidonic acid metabolism

By exerting a tocolytic action on the myometrium,[69] progesterone plays a role in the maintenance of uterine quiescence throughout pregnancy.

Csapo implicated the relative loss of progesterone effect in the mechanism of human parturition.[70] During labor, we observed increasing prostaglandin sensitivity and a decreasing progesterone sensitivity of peripheral lymphocytes.[71] Prostaglandins produced in large quantities during parturition stimulate myometrial contractility. Progesterone both directly and via PIBF prevents prostaglandin F2alpha synthesis and release, thereby promoting uterine quiescence. The above effect of PIBF is abrogated in the presence of exogenous AA, suggesting that PIBF interferes with either the release or the action of AA.[43] Indeed, PIBF inhibits phosholipase A2, (the enzyme needed for the liberation of AA),[72] thus reduces the availability of the precursor for PG synthesis. In the absence of progesterone, prostaglandin F2 alpha increases peripheral NK activity *in vitro*.[73] Neutralization of PIBF in pregnancy lymphocytes results in an increased expression of IL-12, and this is counteracted by simultaneous indomethacin treatment.[74] Luchetti et al.[75] demonstrated an inverse relationship between PIBF and COX2 expression in the implantation sites from DHEA-treated pregnant mice and showed that embryo resorption was related to PIBF deficiency.

Low-dose aspirin treatment of women at risk for pre-term delivery resulted in a decreased peripheral NK activity and a lower rate (11% versus 69% in controls) of pre-term delivery.[76]

Taken together, these data support the concept that by inhibiting AA liberation, PIBF keeps prostaglandin synthesis at a moderate level and via this controls both myometrial contractility and peripheral NK activity during pregnancy (Fig. 2).

The effect of PIBF on NK activity

In vivo data support the effect of PIBF on NK activity. Adoptive transfer of high NK activity spleen cells to pregnant mice, increases fetal loss,[77] and this is corrected by PIBF treatment.[78] Increased resorption rates observed in PIBF-depleted mice are inhibited by treating the mice with anti-NK antibodies,[79] suggesting that PIBF contributes to the success of murine gestation by controlling NK activity.

Both progesterone and PIBF have been shown to inhibit perforin exocytosis and to decrease cytotoxic-

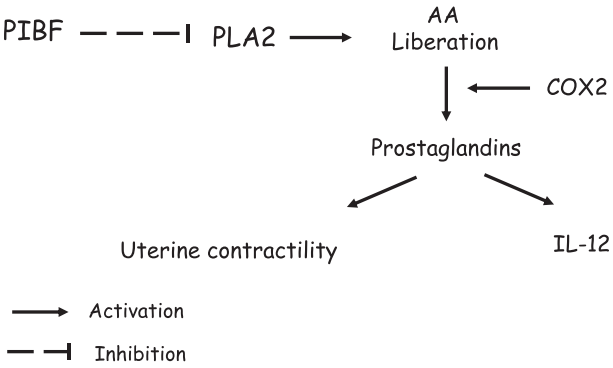

Fig. 2 The effect of progesterone-induced blocking factor on arachidonic acid metabolism.

ity by decidual lymphocytes.[80] Anti-PIBF antibodies reversed the progesterone-mediated reduction in cytolytic activity of decidual lymphocytes. Because 60% of decidual lymphocytes were both CD56+ and PIBF+,[81] these findings suggest that PIBF might mediate the effects of progesterone in regulating cytolytic activity of decidual lymphocytes at the maternal-fetal interface.

PIBF in pregnancy

There is ample evidence that PIBF plays a role in the maintenance of pregnancy. Increased resorption rates because of high NK activity[78] or to progesterone receptor block[82] are precluded by PIBF treatment in mice. On the other hand, neutralization of endogenous PIBF with a specific antibody or inhibiting PIBF synthesis by blocking PR results in a Th1-dominant splenic cytokine production significantly increased NK activity and fetal loss.[83] All the above-mentioned effects are corrected by simultaneous treatment with anti-NK antibodies,[79] suggesting that in mice PIBF is needed for maintaining pregnancy.

In humans, the trophoblast is one of the sources of PIBF.[84] PIBF can be detected in the serum and urine samples of pregnant women. During normal but not in failing pregnancy, the concentration of PIBF continuously increases until the 37th gestational week, followed by a sharp decrease after the 41st week of gestation.[85] PIBF concentrations were significantly lower in urine of patients with pre-eclampsia, than in normal pregnancy and showed a correlation with the number of symptoms presented. In line with this, Check et al.[86] reported that the failure to detect PIBF at 3–5 weeks of seemingly normal pregnancies is associated with a higher miscarriage rate. The same group demonstrated a difference in the percentage of PIBF+ lymphocytes between pregnant and non-pregnant women,[87] and an increased percentage of PIBF+ cells following lymphocyte immunotherapy.[88] Mifepristone treatment for non-surgical pregnancy termination resulted in a decreased percentage of PIBF-positive lymphocytes.[89] On the other hand, dydrogesterone treatment of women with threatened miscarriage proved to be beneficial, via increasing serum PIBF production.[55]

These data, in line with previous *in vivo* findings, suggest that PIBF production is a characteristic feature of normal pregnancy, and determination of PIBF concentration in urine might be of use for diagnosing certain forms of threatened pre-mature pregnancy termination.

Repeated miscarriage can induce anxiety and even depression, which might contribute to further miscarriages. In many case–control trials, the reported high spontaneous success rate in the untreated group might simply be the result of 'tender care'. Psychosocial stress has been shown to alter cytokine production by peripheral lymphocytes of pregnant women during the first trimester of pregnancy. Coussons-Read et al.[90] showed a positive correlation between stress level of the patients and IL-6 production of their lymphocytes, whereas IL-10 production was inversely related to the stress score. The effect of stress on pregnancy outcome has been demonstrated by Arck et al.[91] in mice. In another study,[57] pregnant mice that had been subjected to acoustic stress had significantly higher resorption rates, lower levels of progesterone and PIBF in plasma, as well as a reduced staining intensity for PR at the foeto-maternal interface, compared to control animals. Dydrogesterone treatment of stressed animals corrected the high resorption rates, increased the concentration of plasma PIBF and the percentage of IL-4-positive decidual immune cells in stressed mice. Galectin-1 treatment of stressed mice prevented the decrease in progesterone and PIBF, while progesterone supplementation restored uterine Gal-1 expression, suggesting a synergy between Galectin-1 and the progesterone-PIBF axis.[92]

The impact of stress on pregnancy outcome was investigated in a prospective study including 1098 women with seemingly normal pregnancies. Psychometric data were documented at recruitment (4th–12th week of gestation), and pregnancy outcomes were registered. Among women with high stress perception, PIBF levels were reduced and subsequent miscarriage was more frequent than among those with low stress perception.[93]

These studies imply that initially normal progesterone concentrations might decrease and compromise pregnancy, any time during gestation, owing to stress.

PIBF and tumors

Progesterone-induced blocking factor was first identified as a progesterone-induced immunomodulatory molecule secreted by pregnancy lymphocytes. RNA expression analyses of several human cell lines with different tissue origin and paired human tumor/

normal tissues, as well as of several PR+ and PR− breast tumors revealed that PIBF mRNA is overexpressed in highly proliferating cells independent of the presence of PR. Immunofluorescence microscopy revealed a centrosomal localization for the full-length PIBF, while a 35-kDa form showed a diffuse cytoplasmic staining.[45] Several proteins involved in tumorigenesis are centrosome associated, and disturbed centrosome function causes unequal segregation of chromosomes. The PIBF gene has been identified on chromosome 13 in the vicinity of breast cancer susceptibility genes.[94] Recent studies,[95,96] revealed the presence of PIBF mRNA in several human leukemia cell lines, some of which even expressed the PIBF protein. The addition of progesterone to the media increased the expression of PIBF and mifepristone downregulated its expression. Mifepristone treatment of mice with spontaneous leukemia increased the length and quality of life (determined by the body conditioning score). Only 11.4% of the mifepristone treated mice, whereas 50% of untreated controls died within 2 weeks of therapy. After 2 weeks, 82% of treated mice and 11% of controls had the highest 'quality of life' score.

Progesterone-induced blocking factor seems to be progesterone induced in pregnancy lymphocytes and constitutively expressed in tumors. The centrosomal localization of the full-length form suggests its possible role in cell cycle regulation. The secreted smaller splice variants act on cytokine synthesis and NK activity and by these effects might inhibit efficient local antitumor immunity (Fig. 3).

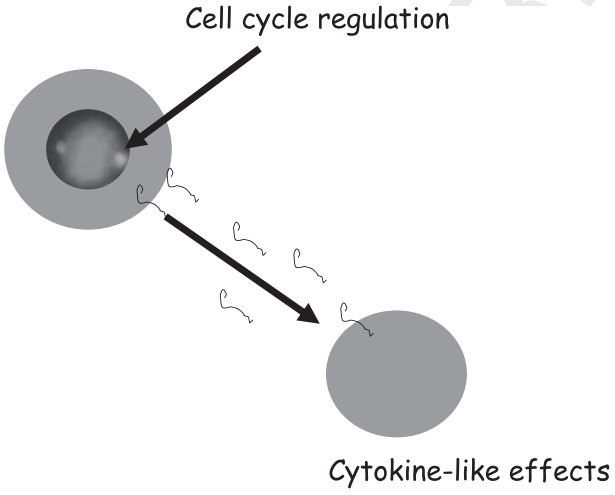

Fig. 3 Possible roles of the centrosome-associated and secreted progesterone-induced blocking factor forms.

Conclusions

Progesterone-induced blocking factor plays a dual role. The centrosomal association suggests an involvement in cell cycle regulation, while the secreted forms act as cytokines. By signaling via the IL-4 receptor, the secreted forms induce a Th2-dominant cytokine pattern, which together with other biological effects of PIBF, establish a Th2-biased immunological environment. PIBF production is an attribute of normal pregnacy, and high PIBF production favors a successful pregnancy. In contrast to this, high PIBF production by a malignant tumor might help tumor escape by suppressing local antitumor immune responses. Blocking PIBF receptors on immunocompetent cells might become a possible therapeutic option for treating malignant tumors.

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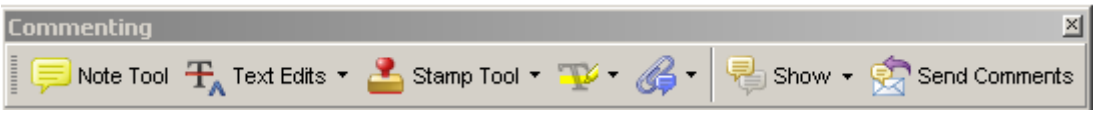

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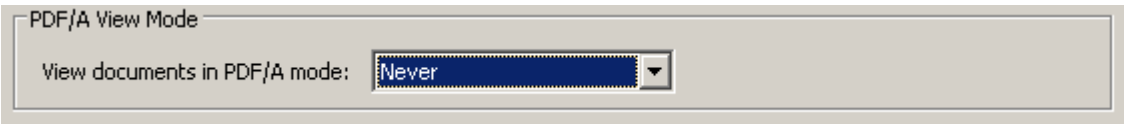

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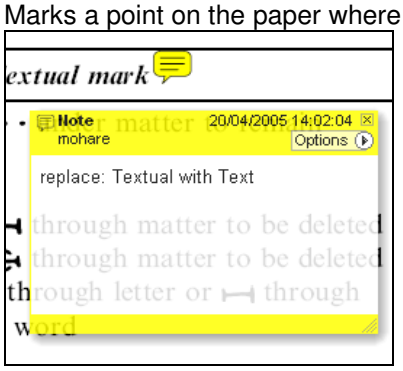

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1. Right click into area of either inserted text or relevance to note
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3. Type comment into the text box
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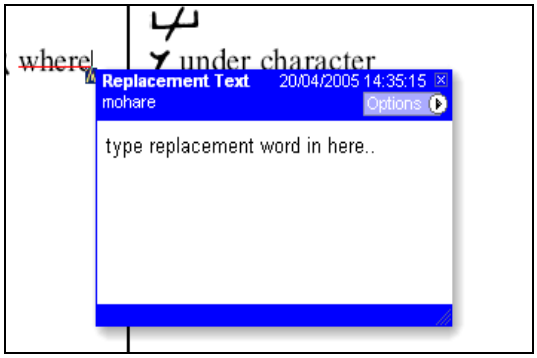
Replacement text tool — For deleting one word/section of text and replacing it

Strikes red line through text and opens up a replacement text box.



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1. Select cursor from toolbar
2. Highlight word or sentence
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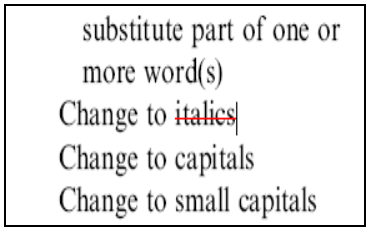
Cross out text tool — For deleting text when there is nothing to replace selection

Strikes through text in a red line.



How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Cross Out Text

Approved tool — For approving a proof and that no corrections at all are required.

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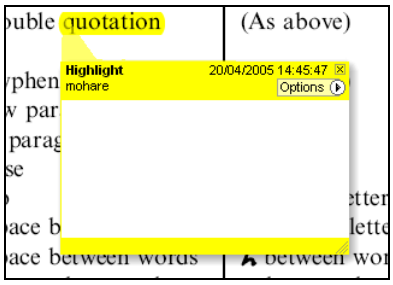
Highlight tool — For highlighting selection that should be changed to bold or italic.

Highlights text in yellow and opens up a text box.



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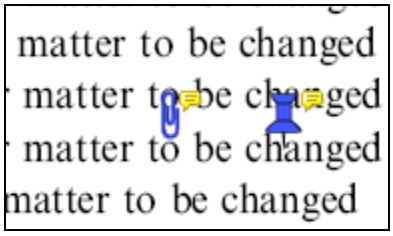
Attach File Tool — For inserting large amounts of text or replacement figures as a files.

Inserts symbol and speech bubble where a file has been inserted.



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1. Click on paperclip icon in the commenting toolbar
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Pencil tool — For circling parts of figures or making freeform marks

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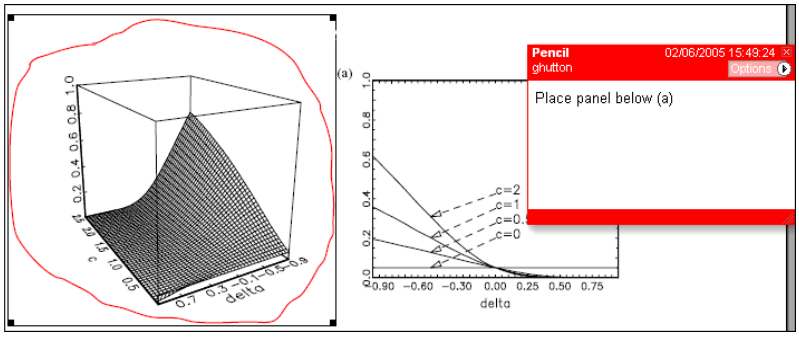

How to use it:

1. Select Tools > Drawing Markups > Pencil Tool
2. Draw with the cursor
3. Multiple pieces of pencil annotation can be grouped together
4. Once finished, move the cursor over the shape until an arrowhead appears and right click
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